Placental phenotype and the insulin-like growth factors: resource allocation to fetal growth

Amanda N. Sferruzzi-Perri[a,1], Ionel Sandovici[b], Miguel Constancia[b], Abigail L. Fowden[a]

[a]Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, Downing Street, University of Cambridge, Cambridge, UK CB2 3EG; [b]Metabolic Research Laboratories, MRC Metabolic Diseases Unit, Department of Obstetrics and Gynaecology and NIHR Cambridge Biomedical Research Centre, Robinson Way, Cambridge, UK CB2 0SW

[1]Corresponding author:
Amanda Sferruzzi-Perri
Centre for Trophoblast Research,
Department of Physiology, Development and Neuroscience,
University of Cambridge,
Cambridge, UK CB2 3EG
Telephone: +44 (0) 1223333807
Email: ans48@cam.ac.uk

Keywords: resource allocation, fetus, placenta, IGF, pregnancy, nutrient transport, signalling
Classification: Integrative
Running title: The IGFs and placental resource allocation to fetal growth

ABSTRACT

The placenta is the main determinant of fetal growth and development *in utero*. It supplies all the nutrients and oxygen required for fetal growth and secretes hormones that facilitate maternal allocation of nutrients to the fetus. Furthermore, the placenta responds to nutritional and metabolic signals in the mother by altering its structural and functional phenotype which can lead to changes in maternal resource allocation to the fetus. The molecular mechanisms by which the placenta senses and responds to environmental cues are poorly understood. This review discusses the role of the insulin-like growth factors (IGFs) in controlling placental resource allocation to fetal growth, particularly in response to adverse gestational environments. In particular, it assesses the impact of the IGFs and their signalling machinery on placental morphogenesis, substrate transport and hormone secretion, primarily in the laboratory species, although it draws on data from human and other species where relevant. It also considers the role of the IGFs as environmental signals in linking resource availability, to fetal growth through changes in the morphological and functional phenotype of the placenta. As altered fetal growth is associated with increased perinatal morbidity and mortality and a greater risk of developing adult-onset diseases in later life, understanding the role of IGFs during pregnancy in regulating placental resource allocation to fetal growth is important for identifying the mechanisms underlying the developmental programming of offspring phenotype by suboptimal intrauterine growth.

KEY POINTS SUMMARY

- Size at birth is critical in determining life expectancy and is dependent primarily on the placental supply of maternal nutrients and oxygen.
- The insulin-like growth factors (IGFs) are important in controlling placental resource allocation to fetal growth during development via their impacts on placental morphogenesis, substrate transport and hormone secretion.
- Placental IGFs (particularly IGF2) alter in response to environmental challenges known to affect placental phenotype and fetal growth.
- IGFs have an important role in optimising fetal growth with respect to resource availability during pregnancy via actions on placental phenotype.

AUTHOR SUMMARY

Amanda Sferruzzi-Perri received her Bachelor of Science degree with Honours and PhD degree from the University of Adelaide, Australia (in 2001 and 2007,respectively). In 2008, she received a CJ Martin Overseas Biomedical Fellowship from the NH&MRC to undertake research at the University of Cambridge, UK. Through the award of a Next Generation Fellowship from the Centre for Trophoblast Research in 2011 and a Dorothy Hodgkin Research Fellowship from the Royal Society in 2014, Amanda has been using a variety of strategies to decipher the role of insulin-like growth factors and their signalling pathway, PI3K in maternal-placental-fetal interactions governing pregnancy success.

INTRODUCTION

Intrauterine growth is a key determinant of lifespan. Babies born growth restricted or large for gestational age are at greater risk of perinatal morbidity and mortality than those of normal birth weight. Moreover, the "memories" of an altered environment and growth *in utero* can stretch beyond the perinatal period to influence health much later in life. Epidemiological studies in humans have shown that babies grown abnormally due to poor maternal nutrition are at heightened risk of developing conditions like type 2 diabetes, heart disease and obesity as adults, and of dying younger as a consequence (Gluckman *et al.*, 2005; Jansson & Powell, 2006). Similarly, manipulating intrauterine growth experimentally by varying maternal food intake, dietary composition, oxygen availability, endocrine status or utero-placental blood flow has been shown to program cardiovascular, metabolic and endocrine function of the adult offspring in a wide range of mammalian species (Gluckman *et al.*, 2005; McMillen & Robinson, 2005; Fowden *et al.*, 2006).

As the interface between the mother and fetus, the placenta is one of the main determinants of intrauterine growth. It supplies all the nutrients and oxygen required for fetal growth as well as secreting hormones that influence maternal metabolism in favour of the fetal needs. Its morphological and functional characteristics, therefore, have an important role in determining the allocation of maternal resources to fetal growth. These characteristics include cell composition, surface area, barrier thickness, blood flow, vascularity, nutrient utilisation and the abundance and activity of the various transporter molecules (Fowden *et al.*, 2009; Sandovici *et al.*, 2012). Recent studies have shown that the placenta can respond to maternal nutritional and metabolic signals by altering these characteristics which, in turn, leads to changes in the placental capacity to supply resources to the fetus (Fowden *et al.*, 2009; Sandovici *et al.*, 2012). Thus, the placenta is a key

mediator in linking maternal environmental conditions to development of the fetus (Burton *et al.*, 2016; Sferruzzi-Perri & Camm, 2016). However, the molecular mechanisms by which the placenta senses and responds to environmental cues during pregnancy are poorly understood. This review discusses the role of the insulin-like growth factors (IGFs) in controlling placental resource allocation to intrauterine growth, particularly in relation to maternal environmental conditions during pregnancy. It focuses primarily on small laboratory animals, like mice, rats and guinea pigs that are most commonly used for these studies but also draws on data from other species, including humans, where available.

THE INSULIN-LIKE GROWTH FACTORS

The insulin-like growth factors (IGFs), IGF1 and IGF2, are 7.5kDa single-chained polypeptides that promote growth, both before and after birth. They affect the metabolism, mitogenesis, survival and differentiation of a wide variety of cell types by binding to IGF receptors (IGF1R and IGF2R), insulin receptor (INSR) and a hybrid IGF1R-INSR receptor with varying affinity (Sferruzzi-Perri *et al.*, 2008; Fernandez & Torres-Aleman, 2012; Harris & Westwood, 2012). Their actions are influenced by at least six different IGF binding proteins (IGFBP-1 to IGFBP-6) and numerous IGF-related binding proteins, which alter access of the IGFs to their receptors and have been reviewed in detail elsewhere (Bach *et al.*, 2005; Bach, 2015; Clemmons, 2016). The main signalling receptor for the IGFs is IGF1R, which activates the phosphoinositide-3 kinase/protein kinase A (PI3K/AKT) and mitogen-activated protein kinase (MAPK) signalling pathways. IGF2 also binds to the IGF2R, which can lead to either IGF2 degradation or activation of the G-protein-coupled signalling pathway (Okamoto *et al.*, 1990).

The *Igf2* gene is subject to parental imprinting and only the paternal allele is expressed. It can be expressed by different promoters, of which P0 (*Igf2P0*) is specific to the placenta in mice (Moore *et al.*, 1997). In mice, though largely not in humans, the *Igf2r* gene is also imprinted but in a reciprocal fashion to *Igf2* with expression from the maternal allele (Monk *et al.*, 2006). The IGFs (particularly IGF2), their receptors and signalling pathways are expressed by the placenta in many species and change in their abundance both developmentally and in response to environmental cues (Sferruzzi-Perri *et al.*, 2010). In many species, circulating IGF concentrations are higher during pregnancy than in the non-pregnant animal and also change in the mother and fetus with proximity to delivery (Fowden, 2003; Sferruzzi-Perri *et al.*, 2010). IGF2 is more abundant than IGF1 in both the maternal

and fetal circulations in all species studied to date, with the exception of mice (Fowden, 2003; Sferruzzi-Perri *et al.*, 2010). IGF2 is also more highly expressed than IGF1 by the placenta in all species studied to date (Sferruzzi-Perri *et al.*, 2010).

THE EFFECTS OF THE INSULIN-LIKE GROWTH FACTORS ON PLACENTAL PHENOTYPE

The effects of the IGFs on the placenta have been studied directly in two main ways. First, they have been given exogenously either to placental cultures *in vitro* or to pregnant animals *in vivo* to study placental growth, transport and endocrine function. Secondly, the *Igf* genes, their receptors and key molecules in their downstream pathways have been under- or over-expressed in genetically modified mice to determine the morphological and functional consequences for the placenta at different stages of pregnancy. While the functions of the placenta are common across species, its structure varies in terms of shape, organisation of trophoblast lineages, extent of invasion into the maternal uterus, and degree of interdigitation at the feto-materno interface (reviewed in depth elsewhere (Carter, 2007; Wooding & Burton, 2008; Roberts *et al.*, 2016)). For instance, the human and non-human primate placenta is composed of a series of highly branched structures, called villi. These contain a mesenchymal core that has fetal capillaries which are closely associated with an overlying syncytiotrophoblast layer. The syncytiotrophoblast is directly bathed in maternal blood and functions in both transport and hormone secretion. Cytotrophoblast cells, can fuse to form the syncytiotrophoblast or migrate from the villous tree into the decidua where they invade and remodel uterine spiral arteries to promote blood flow to the placenta. The syncytiotrophoblast is also bathed in maternal blood in the mouse, rat and guinea pig placenta. However, the mouse placenta is arranged into two morphologically and functionally distinct regions; the labyrinth zone (Lz) that is responsible primarily for transport and the junctional zone (Jz; also known as basal or interlobium region) which functions in uterine remodelling/invasion and hormone secretion. In ruminate species like the sheep and cow the placenta is comprised of individual placentomes which form at specialised sites called caruncles, in the uterine wall. The overlaying trophoblast layer can be a syncitium (in sheep) or remains uni-cellular (columnar epithelium; in cows) and there no invasion of the maternal blood vessels by trophoblast cells. However, in sheep some trophoblast cells migrate and fuse with caruncle epithelial cells and play an endocrine role.

Exogenous administration of IGFs

In vitro experiments

IGF1 and IGF2 prevent apoptosis and enhance proliferation and migration/invasion of human placental villous explants, primary trophoblast cultures and trophoblast cell lines from the first trimester and term (Table 1). IGF1 also promotes the proliferation, invasion and survival of first trimester human placental fibroblasts (Miller *et al.*, 2005) and the differentiation of term trophoblast cells into syncytiotrophoblast (Bhaumick *et al.*, 1992; Milio *et al.*, 1994; Cohran *et al.*, 1996). Similarly, IGF1 stimulates proliferation and migration of murine ectoplacental cone trophoblast in culture (Kanai-Azuma *et al.*, 1993) and early pregnancy porcine trophoblast cells (Jeong *et al.*, 2014). Furthermore, IGF2 promotes differentiation of murine ectoplacental cone trophoblast and migration of ovine trophoblast cells *in vitro* (Kim *et al.*, 2008). Using receptor and pathway inhibitors and IGF analogues with selectivity for particular receptors, some of the molecular mechanisms mediating the actions of IGFs on the human placenta have begun to be identified *in vitro*. IGFs appear to mediate their proliferative and anti-apoptotic effects on trophoblast through activating IGF1R and triggering the MAPK and PI3K/AKT signalling pathways, respectively (Forbes *et al.*, 2008). IGFs also induce trophoblast migration and invasion through IGF1R, and possibly INSR with subsequent activation of MAPK and PI3K/AKT signalling pathways (Diaz *et al.*, 2007; Shields *et al.*, 2007; Forbes *et al.*, 2008; Mayama *et al.*, 2013). However, IGF2 may also signal via IGF2R and G_i proteins, MAPK and Rho GTPase pathways to trigger trophoblast migration and invasion (McKinnon *et al.*, 2001; Shields *et al.*, 2007; Harris *et al.*, 2011). Thus IGFs promote the growth of different cell lineages in the placenta via multiple mechanisms (Figure 1A).

In addition to stimulating placental growth, both IGFs stimulate glucose and System A amino acid uptake and IGF1 increases System L activity but reduces lipoprotein lipase activity in human trophoblast *in vitro* (Table 1). However, these changes in nutrient uptake do not always track with the expression of the transporter genes or proteins, suggesting that the IGFs may also affect post-transcriptional/translational mechanisms (Fang *et al.*, 2006; Jones *et al.*, 2013; Jones *et al.*, 2014). Indeed, IGF1 was recently shown to stimulate glucose transporter capacity by increasing the translocation of GLUT1/SLC2A1 to the trophoblast plasma membrane (Baumann *et al.*, 2014). In culture, IGF1 prevents the release of the vaso-constrictors, prostaglandin E and F, and thromboxane, by the term human placenta and reduces the agonist-mediated vasoconstriction of human myometrial arteries (Siler-Khodr *et al.*, 1995; Corcoran *et al.*, 2012). *In vivo*, these effects could increase utero-placental blood flow and substrate transfer in late gestation. Both IGF1 and IGF2 also enhance trophoblast endocrine capacity in culture. IGFs increase the secretion of hormones including progesterone, human chorionic gonadotrophin and placental lactogen *in vitro* although

192 others, like placental growth hormone may not be affected (Maruo *et al.*, 1995; Zeck *et al.*, 2008;
193 Rak-Mardyla & Gregoraszczuk, 2010). In addition, IGF2 simulates the differentiation of hormone-
194 producing murine and ovine trophoblast *in vitro* (Kanai-Azuma *et al.*, 1993; Kim *et al.*, 2008). Thus,
195 IGFs have the capacity to promote growth, hormone secretion and substrate transport capacity of
196 the placenta.
197
198
199 ***In vivo experiments***
200 Treatment of guinea pig dams with either IGF1 or IGF2 in early-mid pregnancy increases fetal weight
201 near term [Table 1; (Sferruzzi-Perri *et al.*, 2006)]. With exogenous IGF1, placental Lz area and *Igf2*
202 gene expression is reduced during the treatment, even though fetal weight is increased already in
203 mid pregnancy (Sohlstrom *et al.*, 2001; Sferruzzi-Perri *et al.*, 2007b; Standen *et al.*, 2015). Whilst
204 there is no sustained effect of either IGF on placental weight, IGF2 increases the volume and surface
205 area of the transport Lz, near term [Table 1, (Sferruzzi-Perri *et al.*, 2006)]. Development of the
206 placental exchange region was further enhanced when the IGF2R-selective synthetic analogue,
207 Leu^{27}-IGF2 was administered maternally (Sferruzzi-Perri *et al.*, 2008). In mice, maternal Leu^{27}-IGF2
208 treatment from day 13 of pregnancy halves the number of fetuses naturally growth-restricted within
209 the litter near term (Charnock *et al.*, 2016). Taken together, these findings suggest that maternal
210 IGF2 in early gestation may act, in part, via the IGF2R to enhance functional development of the
211 placenta with beneficial impacts on fetal growth. However, caution is warranted as part of the
212 effects of Leu^{27}-IGF2 could be due to the displacement of endogenous IGF2 and its subsequent
213 interaction with IGF1R and INSR in the placenta.
214
215 Exogenous IGFs also modify the functional capacity of the placenta to supply resources for fetal
216 growth. In the late pregnant ewe, increasing IGF1 in the fetal circulation increases amino acid and
217 glucose uptake by the placenta but may reduce materno-fetal transfer of these substrates, lactate
218 production and the number of placentomes (Table 1). Increasing IGF1 in the maternal circulation
219 also alters placental metabolic function in the pregnant ewe near term; glucose transfer capacity
220 and lactate production are enhanced by an acute infusion of IGF1 (Liu *et al.*, 1994). In guinea pigs,
221 placental delivery of glucose and/or neutral amino acids to the fetus is increased in late gestation by
222 chronic maternal IGF treatment in early-mid pregnancy (Table 1). This enhanced placental transfer in
223 late gestation is partly due to increased expression of nutrient transporters (System A amino acid;
224 *SNAT2/Slc38a2*) by IGF1 in mid pregnancy and improved development of the exchange region by
225 IGF2 in late pregnancy (Sferruzzi-Perri *et al.*, 2006; Sferruzzi-Perri *et al.*, 2007b). In mice, the

variability in System A amino acid transport capacity and conceptus weight within the litter is abolished by maternal Leu^{27}-IGF2 (Charnock *et al.*, 2016) and data suggest that IGFs may have most benefit for improving growth of the smallest pups. Indeed, maternal Leu^{27}-IGF2 improves the weight of fetuses that are growth restricted due to a lack of the endothelial nitric oxide gene and reduces the number of pups below the fifth centile of the wild-type population in late gestation (Charnock *et al.*, 2016). In addition to improving placental transport function, exogenous IGFs also affect endocrine capacity *in vivo* (Figure 1B). Maternal IGF2 treatment simulates the development of the endocrine Jz of the rat placenta (Van Mieghem *et al.*, 2009) and exogenous IGF1 and IGF2 increase placental pro-renin activation in guinea pigs (Standen *et al.*, 2015). Thus, IGFs may also increase fetal resource supply through changing placental endocrine function and thus maternal adaptations to pregnancy, however further studies are warranted.

To circumvent possible confounding effects of systemic IGF treatment on the mother, approaches are being developed to target IGFs to the placenta. In mice, adenoviral-mediated site-specific intraplacental transfer of the *Igf1* gene on day 14 of pregnancy, increases the area of the placenta, the size of the Lz and of the maternal and fetal facing areas three days later, although there is no change in conceptus weight [Table 1; (Katz *et al.*, 2009)]. In response to liposome-mediated targeting of IGF2 to the mouse placenta, placental growth is also increased although fetal weight is not affected (King *et al.*, 2016). In rabbits, the weight of natural runt fetuses in the litter is increased two days following placental *Igf1* transgene delivery without a change in placental weight however how it impacts on structure and function of the placenta remains unknown (Keswani *et al.*, 2015). These data suggest that targeting of IGF delivery to the placenta may prove an effective method of improving placental function and, thus, fetal growth, particularly when feto-placental growth is impaired.

Genetic manipulation of the IGF system

In mice, knockout of the *Igf2* gene in the entire conceptus or predominantly within the fetal or trophoblast cell lineages leads to placental and fetal growth restriction, with the greatest reduction in growth seen with ubiquitous *Igf2* loss (Table 2). Similarly, a heterozygous deficiency in the PI3K-p110α (*Pik3ca*; homozygous deficiency is lethal) or complete ablation of the AKT1 (*Pkba*) or MAPK1 (*Erk2*) genes, causes feto-placental growth restriction (Cho *et al.*, 2001; Hatano *et al.*, 2003; Yang *et al.*, 2003; Yung *et al.*, 2008; Kent *et al.*, 2012; Sferruzzi-Perri *et al.*, 2016). In contrast, over-expressing the *Igf2* gene through activating the normally silent maternal gene copy in the *H19* null, increasing

IGF2 availability via *Igf2r* ablation, or deletion of the PI3K signalling inhibitor (*Pten*), results in over-growth of the fetus and placenta (Leighton *et al.*, 1995; Ludwig *et al.*, 1996; Louvi *et al.*, 1997; Ripoche *et al.*, 1997; Church *et al.*, 2012). Deletion of the *Igf1, Igf1r* or *Insr* genes in mice also leads to fetal growth restriction, but placental weight is unaffected (DeChiara *et al.*, 1990; Baker *et al.*, 1993; Louvi *et al.*, 1997). This suggests that the growth-promoting effect of IGF2 in the mouse placenta occurs independently of IGF1R and INSR, possibly through an unknown, distinct placental-specific receptor (XRp) (Louvi *et al.*, 1997). However, evidence from *H19* null mutants suggests that IGF1R could contribute to the control of placental growth in mice as the first exon of the *H19* gene encodes *miR-675* which targets *Igf1r* for reduced expression (Keniry *et al.*, 2012). Overgrowth of the *H19* null placenta (Leighton *et al.*, 1995; Esquiliano *et al.*, 2009; Angiolini *et al.*, 2011; Church *et al.*, 2012), is thus thought to be due to biallelic *Igf2* via imprinting mechanisms, as well as, enhanced *Igf1r* expression through loss of *miR-675* (Keniry *et al.*, 2012). Taken together, these data highlight the importance and complexity of the IGF system in controlling conceptus growth in mice.

Genetic manipulations of *Igf2, Igf2r* and the downstream signalling pathways also affect the morphology of the placenta (Table 2). For instance, loss of *Igf2* (complete and *Igf2P0* null)*, Pik3ca, Pkba* or *Erk2* gene expression causes defective Lz formation. In particular, Lz volume/thickness, exchange surface area and vascularisation are all reduced and the interhaemal barrier to diffusion of gases like oxygen is greater in the placenta of all these mutants (Table 2). In contrast, in the *H19* null, the Lz surface area is increased in line with the placentomegally observed (Angiolini *et al.*, 2011). In addition, IGF2 affects the formation of endocrine cells in the placenta. In particular, loss or gain of *Igf2* or the PI3K-AKT signalling pathway causes a disproportionate decrease or expansion of the glycogen cells in the Jz, whereas *Igf1r* or *Insr* nulls show no changes in Jz glycogen cell abundance (Table 2). Collectively, the available data suggest that IGF2 acts via both the PI3K/AKT and MAPK pathways to attain normal placental weight and Lz structure, and through PI3K/AKT signalling to drive placental glycogen cell formation in mice (Figure 2A).

Placental function also changes when the IGF system is genetically modified in mice (Table 2). The passive permeability of the placenta to hydrophilic nutrients/solutes is reduced in the complete *Igf2* null, placental-specific *Igf2P0* null and *H19* null (Constancia *et al.*, 2002; Sibley *et al.*, 2004; Coan *et al.*, 2008b; Angiolini *et al.*, 2011). The complete *Igf2* null placenta transports less neutral amino acid (methyl amino-isobutyric acid, MeAIB) via the System A transporters in association with reduced *SNAT2/Slc38a2* expression (Constancia *et al.*, 2005). There is also reduced abundance of System X_{AG}^- and System Y^+ transporters, responsible for placental transfer of cationic and anionic amino acids, in

294 the complete *Igf2* and the *Igf1r* null (Matthews *et al.*, 1999). In contrast, the *Igf2P0* null placenta
295 transports more neutral amino acids via System A, as well as, more glucose and calcium in late
296 gestation (Table 2). Up-regulation of placental transport capacity is associated with increased
297 expression of *SNAT4/Slc38a4* and *GLUT3/Slc2a3* by the *Igf2P0* deficient placenta. In contrast to *Igf2,*
298 there is little or no information on the capacity of the *Igf1* or *Insr* null placenta to supply nutrients to
299 the fetus. In the complete *Igf2* null, placental and fetal growth restriction occurs concurrently and
300 becomes evident in mid-gestation [Table 2; (Baker *et al.*, 1993; Constancia *et al.*, 2005)]. In the
301 *Igf2P0* null, placental weight is reduced at a similar time in gestation, but fetal growth only becomes
302 restricted much closer to term and to a lesser extent than in the complete *Igf2* null (Baker *et al.*,
303 1993; Constancia *et al.*, 2002; Constancia *et al.*, 2005). Liposome-mediated targeting of IGF2 to the
304 placenta has recently been shown to increase the weight of *Igf2P0* null mouse fetuses near term
305 [Table 1; (King *et al.*, 2016)]. Collectively, these findings suggest that the *Igf2P0* null placenta
306 compensates for its defective development and compromised permeability by adaptively up-
307 regulating its nutrient transport systems and thereby, minimises the degree of fetal growth
308 restriction, relative to the complete *Igf2* null. The *Pik3ca* heterozygote deficient placenta also
309 transfers glucose and amino acids via System A transporters with increased efficiency in
310 compensation for its impaired development, which is associated a less severe reduction in fetal
311 weight close to term than earlier in gestation (Sferruzzi-Perri *et al.*, 2016). Moreover, the naturally
312 small placenta that supports more fetal mass per gram shows increased expression of *Igf2P0*
313 coupled with a preservation of Lz growth and with increased placental System A transport capacity
314 and *SNAT2*/*Slc38a2* abundance compared to the large placenta in the litter (Coan *et al.*, 2008a). In
315 contrast, the over-grown *H19* null placenta shows diminished neutral amino acid and glucose
316 transport which is thought to limit fetal over-growth and avoid an excessive drain of maternal
317 resources into the fetus (Angiolini *et al.*, 2011). Thus, IGF2 in the placenta is important for fine-
318 tuning nutrient supply to the fetus (Figure 2B).
319
320 In addition to effects on placental transport, the *Igf2* gene may also affect the endocrine function of
321 the placenta with consequences for maternal physiology during pregnancy. Evidence for this stems
322 from associations between altered placental Jz formation in *H19* and *Igf2P0* null mutants and raised
323 circulating glucose, insulin and/or corticosterone in phenotypically wild-type dams (Petry *et al.*,
324 2010; Sferruzzi-Perri *et al.*, 2011). Thus, IGF2 has an important role in nutrient allocation to the
325 fetus. By regulating placental phenotype, it balances the fetal genetic drive for growth with the
326 maternal ability to supply the required resources, thereby optimising both offspring and maternal
327 fitness.

IGFS AS ENVIRONMENTAL SIGNALS IN REGULATING PLACENTAL RESOURCE ALLOCATION TO FETAL GROWTH

IGFs may also play an important role in changing placental resource allocation to the fetus in environmentally-challenged pregnancies. As *Igf1* expression is relatively low in the placenta, studies have largely focussed on placental expression of *Igf2* and activation of its signalling pathways (Table 3). However, since the signalling pathways are responsive to both IGFs, the placenta can also respond to changes in circulating IGF1 and IGF2 induced by nutritional or other environmental cues.

Maternal nutrition

Undernutrition

In mice, guinea pigs and baboons, undernutrition restricts placental growth in association with a decrease in the expression of *Igf2* and/or signalling via the PI3K/AKT and MAPK pathways (Table 3). There are also reductions in placental vascularisation, exchange surface area, Jz volume and glycogen cell abundance and/or a greater barrier to diffusion with maternal undernutrition in mice and guinea pigs; morphological parameters that were altered similarly by a genetic deficiency in *Igf2, Pik3ca, Pkb* and *Erk2* (Tables 2 and 3). Together, these studies suggest that decreases in IGF2 expression and signalling within the placenta could underlie the growth and morphological defects observed with maternal undernutrition in these species. In larger animals, the expression of *Igf2* and its signalling machinery reduces, is unchanged or even increases in response to undernutrition (Table 3). For instance, signalling via MAPK and PI3K/AKT in the placenta is up-regulated in nutrient-restricted ewes and hiefers (Zhu *et al.*, 2007a; Zhu *et al.*, 2007b; Ma *et al.*, 2011). In these models, changes in signalling relate to a normalisation of placental weight or an increase in placental cotyledon vascularity. They also correlate with a maintenance or restoration of fetal weight in later gestation, despite an exposure to undernutrition. In ewes of a moderate condition, which have the smallest placentas supporting more mass of fetus per gram, placental expression of *Igf2* is greatest (Osgerby *et al.*, 2003). These studies therefore suggest that in larger species, there is morphological adaptation of the placental to an adverse maternal nutritional state through increasing *Igf2* and growth signalling locally.

In the undernourished sheep placenta with increased PI3K/AKT and MAPK signalling, the expression of glucose and fatty acid transporters is also increased (Ma *et al.*, 2011). However, in undernourished baboons, diminished *Igf2* expression and signalling in the placenta accompanies reductions in Systems A and L amino acid transporter capacity and glucose transporter gene expression (Pantham *et al.*, 2015; Pantham *et al.*, 2016). Taken together, these studies suggest that IGF2 and the PI3K/AKT and MAPK signalling pathways could also mediate changes in placental transport function during undernutrition. In mice, despite a 20% reduction in maternal food intake and placental growth restriction earlier in gestation, fetal weight is normal until just prior to term (Coan *et al.*, 2010). This maintenance of fetal growth relates to an initial preservation of Lz development in earlier gestation and an adaptive up-regulation of System A amino acid transporter capacity and *SNAT2/Slc38a2* expression near term, by the growth restricted undernourished placenta. However, in mice lacking the placental-specific *Igf2* isoform (*Igf2P0*) these adaptations to maternal undernutrition fail to occur. The development of the placental exchange region is compromised earlier in gestation, there is no up-regulation of amino acid transport or *SNAT2/Slc38a2* expression and reduced *SNAT4/Slc38a4* abundance near term in *Igf2P0* null placentas compared to wildtype in undernourished mice (Sferruzzi-Perri *et al.*, 2011). As a result, fetal growth is restricted earlier in gestation and more adversely affected near term by undernutrition, in *Igf2P0* nulls. The *Igf2P0* transcript is, therefore, a major determinant of the environmental modification of placental phenotype with undernutrition in mice. The expression of genes involved in glucose, neutral amino acid and fatty acid transport, as well as, the IGF signalling pathways in the human placenta are modified by the diet and physical activity of the mother during pregnancy (Brett *et al.*, 2015). Thus, the IGF system may also be important for modifying resource capacity of the human placenta in response to changes in the maternal environment.

Low-protein diets

During rodent pregnancy, consumption of an iso-calorific low protein diet has inconsistent impacts on both placental weight and placental *Igf2* expression [Table 3 and (Sferruzzi-Perri & Camm, 2016)]. However, the nature of the specific effect appears to depend on the degree of protein deprivation, stage of pregnancy studied and sex of the conceptus (Jansson *et al.*, 2006; Coan *et al.*, 2011; Nusken *et al.*, 2011; Gao *et al.*, 2012a). Despite the contrasting results, placental *Igf2* expression seems to track positively with the weight of the placenta in mice and rats (Coan *et al.*, 2011; Nusken *et al.*, 2011; Gao *et al.*, 2012a). For instance, in pregnant mice, low protein diets cause placentomegaly and the degree of placental weight increase relates to the level of *Igf2* up-regulation at first appearance

of growth enhancement (Coan *et al.*, 2011). The variation in placental growth and *Igf2* expression observed in different models of protein deficiency could be caused by the content and source of carbohydrate used to maintain calorie intake. Nevertheless, taken together, these findings suggest that at least part of the changes in placental growth seen with protein deprivation could be mediated through local changes in *Igf2*.

There are also changes in placental transport capacity with gestational protein malnutrition. For instance, in response to a diet with 8% protein, the mouse placenta adaptively transports more glucose to the fetus on day 16 of pregnancy (Coan *et al.*, 2011). This up-regulation occurs when placental *Igf2* expression is also increased and when fetal growth is maintained despite maternal protein deprivation (Coan *et al.*, 2011). A few days later however, glucose transport is unchanged, System A amino acid transporter abundance is reduced and *Igf2* expression no longer increased in the placenta by a low protein diet, and fetal growth restriction ensues (Coan *et al.*, 2011). These data suggest that placental *Igf2* may be important for adapting nutrient supply to the fetus in response to maternal protein malnutrition in mice. However, there is evidence that pathways downstream of *Igf2* may also be important. For instance, the mechanistic target of rapamycin (mTORC1) mediates the mitogenic and metabolic actions of IGFs (Jansson *et al.*, 2012b). In rats, protein deprivation reduces mTORC1 signalling, Systems A and L amino acid transport and *SNAT2/Slc38a2, LAT1/Slc7a5* and *LAT2/ Slc7a8* expression by the placenta, prior to the appearance of placental and fetal growth restriction (Jansson *et al.*, 2006; Rosario *et al.*, 2011). These findings suggest that down-regulation of signalling pathways like mTORC1 and amino acid transporters in the placenta could link maternal protein restriction to decreases in fetal growth. The availability of protein and specific amino acids during pre-implantation rodent development is linked to alterations in the expression of genes within the *H19-Igf2* locus, mTORC1 signalling and trophoblast cell formation and differentiation with consequences for feto-placental phenotype in late gestation (Kwong *et al.*, 2006; Van Winkle *et al.*, 2006; Eckert *et al.*, 2012; Watkins *et al.*, 2015). Thus, changes in *Igf2* expression and its signalling pathways could be responsive to the availability of nutrients from the earliest stages of development.

Diets with excess sugar and/or fat

The expression of *Igf2* and its signalling pathways in the placenta are inconsistently altered by diets with excess sugar and/or fat (Table 3). Weight of the conceptus may also be reduced, increased or unchanged, depending on the level of fat in the diet, the amount of simple sugars consumed and the

timing of the dietary manipulation [Table 3 and reviewed in (Sferruzzi-Perri & Camm, 2016)]. Part of these variations in *Igf2* expression and conceptus growth could be due to the differences in protein and micronutrient intake, as species like mice and rats control their calorie intake tightly (Keesey & Hirvonen, 1997). In mice fed a diet containing 2.5-times the fat of the controls, placental weight is reduced in early pregnancy in association with decreases in the expression of *Igf2* and signalling machinery, including *Mtor* (Sasson *et al.*, 2015). These placental changes accompanied reductions in the expression of System A amino acid transporter, *SNAT1/Slc38a1*, glucose transporter *GLUT1/Slc2a1* and/or fatty acid translocase, *CD36* depending on the length of high fat feeding and whether the diet was eaten before pregnancy (Sasson *et al.*, 2015). In over-nourished ewes, placental weight is reduced in mid-gestation in association with decreased activity of the IGF signalling pathway (including activation of IRS1 and mTORC1) and changes in vessel size and density in the placenta (Zhu *et al.*, 2009; Ma *et al.*, 2010). However, fetal weight is increased along with fatty acid transporters and translocases in the placenta, suggesting that alternative signalling pathways may be activated to adapt placental nutrient supply to the fetus in ewes with excess food intake (Zhu *et al.*, 2010; Tuersunjiang *et al.*, 2013).

In other studies, increases in the placental IGF system are coupled with improved placental resource allocation to the fetus in dams fed obesogenic diets (King *et al.*, 2013; Sferruzzi-Perri *et al.*, 2013; Diaz *et al.*, 2015; Rosario *et al.*, 2015; Rosario *et al.*, 2016). For instance, in mice, consumption of a high sugar and fat diet from day 1 of pregnancy initially causes conceptus growth restriction and morphological defects in the placental Lz. However, fetal weight normalises by term, despite the persistence of placental growth and morphological defects through adaptive up-regulation of glucose and neutral amino acid transport to the fetus by the placenta (Sferruzzi-Perri *et al.*, 2013). Up-regulation of transport capacity relates to increased expression of *GLUT3/Slc2a3* and *SNAT2/Slc38a2,* as well as, elevated expression of the placental-specific *Igf2* isoform and PI3K/AKT signalling in the placenta in dams fed a diet with excess sugar and fat. Obesogenic diets fed from before pregnancy also increase placental nutrient transporter capacity (glucose, Systems A and L amino acid and fatty acids) in line with greater *Igf2* or PI3K/AKT and mTORC1 signalling, however responses varied with the precise composition of the diet and possibly, fetal sex (King *et al.*, 2013; Aye *et al.*, 2015; Diaz *et al.*, 2015; Rosario *et al.*, 2015; Rosario *et al.*, 2016). The expression of IGF signalling machinery (receptors, AKT, mTORC1) and nutrient transporters is also altered in the placenta from obese women, however, the specific nature of these changes appears to depend on the level of maternal body fat mass, gestational weight gain and whether macrosomia is observed (Jansson *et al.*, 2012a; Brett *et al.*, 2016; Martino *et al.*, 2016). Taken together, these findings suggest

that obesity and obesogenic diets alter placental phenotype in association with changes in placental *Igf2* system and fetal growth.

Maternal hypoxia

In mice, hypoxia typically reduces fetal growth in a severity-dependent manner without a change in placental weight [Table 3 and reviewed in (Sferruzzi-Perri & Camm, 2016)]. However, if the hypoxic challenge commences early in pregnancy, placentomegaly is observed in associated with greater maternal blood spaces and activation of the PI3K/AKT and mTORC1 signalling pathways in the placenta (Matheson *et al.*, 2015). Even though placental weight may not be altered when maternal hypoxia commenced later in pregnancy, placental expression of the IGF system and capacity to supply resources to the fetus is altered (Table 3). In particular, placental expression of IGF receptors, INSR and PI3K isoforms is decreased in response to five days of 13%-10% maternal hypoxia in late mouse gestation, and in 10% hypoxia this effect is due to reductions in maternal food intake (Cuffe *et al.*, 2014; Higgins *et al.*, 2015). However, expression of *Igf2, Igf2P0* and activated AKT increases with 13% hypoxia, but is unchanged or even decreased in response to 5 days of 12-10% hypoxia near term (Cuffe *et al.*, 2014; Higgins *et al.*, 2015). In the 13% hypoxic mouse placenta showing increases in IGF2 expression and signalling, there are beneficial changes in Lz structure including improved vascularisation, maternal blood spaces and a thinner diffusion barrier to exchange; changes that would optimise oxygen delivery to the fetus near term (Higgins *et al.*, 2015; Matheson *et al.*, 2015). There is also greater placental glucose uptake and transport and maintained delivery of neutral amino acids to the fetus when 13% hypoxia occurs in the last third of pregnancy (Higgins *et al.*, 2015). In contrast, in the 12-10% hypoxic placenta with unchanged or decreased expression of the IGF2 system, the morphology of the placental Lz is compromised, with reductions in maternal blood spaces and surface area and a greater barrier to diffusion; changes that would further limit fetal oxygen supply in hypoxic dams (Cuffe *et al.*, 2014; Higgins *et al.*, 2015). Moreover, placental glucose uptake and transport capacity is not up-regulated or even reduced (less *GLUT1/Slc2a1*) and delivery of neutral amino acids diminished, in dams exposed to 12-10% hypoxia, depending on whether food intake is reduced and the sex of the fetus (Cuffe *et al.*, 2014; Higgins *et al.*, 2015). In culture, 1% hypoxia reduces the outgrowth of mouse ectoplacental cone trophoblast in association with diminished *Igf2* expression (Pringle *et al.*, 2007). Hypoxia (1% oxygen) also diminishes the expression of PI3K/AKT and mTORC1 signalling in human trophoblast cell lines (Yung *et al.*, 2012a) and modulates IGF1 and IGF2 signalling in early pregnancy placental mesenchymal stem cells (Youssef *et al.*, 2014; Youssef & Han, 2016). Placental expression of the PI3K/AKT and mTORC1 signalling

pathways and *GLUT1/Slc2a1* expression are decreased in women at 3100m above sea level who deliver growth-restricted babies (Zamudio *et al.*, 2006; Yung *et al.*, 2012a). In addition, inducing endoplasmic stress in the mouse placental Jz genetically is associated with defects in PI3K/AKT and mTORC1 signalling, altered IGF2 glycosylation and bioactivity, and with feto-placental growth restriction (Yung *et al.*, 2012b). Taken together, these findings suggest that activating IGF2 and/or PI3K/AKT signalling in the placenta may be critical for adapting placental resource allocation to the fetus during hypoxia in late pregnancy. They also suggest that the placenta may integrate signals of oxygen and nutrient availability through the IGF2 system to adapt its phenotype and optimize maternal resource supply to fetal growth. Indeed, the mouse *Igf2* gene harbours a hypoxia-responsive element in its promoter (Feldser *et al.*, 1999), as well as CHORE motifs, which bind the glucose-responsive transcription factor, MLX (Hunt *et al.*, 2015). Therefore, the availability of oxygen and nutrients *in utero* could have direct effects on placental *Igf2.* Nutritional and hypoxic challenges alter the concentration of hormones like the glucocorticoid stress hormone and insulin, in the maternal circulation (Sferruzzi-Perri *et al.*, 2011; Cuffe *et al.*, 2014). Thus, changes in placental phenotype may reflect alterations in the metabolic and endocrine state of the mother.

Maternal endocrine challenges

Endocrine challenges can affect maternal metabolism and utilisation of nutrients and thus the partitioning of resource to the conceptus in pregnancy (Vaughan *et al.*, 2011). Administering corticosterone or the synthetic glucocorticoid, dexamethasone to rodents for 3-7 days reduces fetal and placental weights during gestation [Table 3 and (Vaughan *et al.*, 2011)]. In mice, corticosterone decreases AKT and mTORC1 activation in association with reductions in feto-placental weight, Lz vascularisation and glucose and System A amino acid transporter capacity, however the specific nature of these effects depend on when in pregnancy the over-exposure occurs (Table 3). Administering the synthetic glucocorticoid, dexamethasone reduces the expression of MAPK and weight of placenta in female, but not male conceptuses and there is no change in glucose and *SNAT/Slc38a* amino acid transporters irrespective of fetal sex (Cuffe *et al.*, 2011). In mice, placental Igf2 expression is unaffected by maternal administration of corticosterone and dexamethasone even though the conceptus may be growth-restricted (Cuffe *et al.*, 2011; Vaughan *et al.*, 2012; Vaughan *et al.*, 2015). Whereas restrain stress increases placental *Igf2* but does not alter offspring weight in mice (Pankevich *et al.*, 2009). In rats, dexamethasone decreases placental *Igf2* and the level of activated AKT, particularly in the endocrine Jz (Ain *et al.*, 2005). In dexamethasone-treated rats, there are reductions in the expression of prolactin-related family genes by the Jz in late gestation, which may influence the maternal adaptations to pregnancy and, thus, alter the fetal supply of

nutrients indirectly (Ain *et al.*, 2005). The expression of *Igf2* by the term human placenta is also altered in women with elevated plasma cortisol during pregnancy due to emotional distress (Mina *et al.*, 2015). Glucocorticoid response elements have been identified in the human *Igf1* gene promoter (He *et al.*, 2016) however, very little is known about whether glucocorticoids could have direct effects on placental *Igf2* expression. Collectively these findings suggest that reductions in placental *Igf2* system and the functional phenotype of the placenta could link elevated maternal glucocorticoids to decreases in fetal growth.

In rats, pre-existing maternal diabetes also alters the expression of the IGF system in the placenta, as well as, materno-fetal resource allocation, however, the direction of change depends on how long the dam was insulin deficient/dysglycemic. For instance, *Igf* expression and IGF1R activation are elevated in association with greater glycerol and free fatty acid transfer by the placenta and an increase in fetal weight by 13% in rats that are diabetic from neonatal life (White *et al.*, 2015). Placental lipid transport capacity is also increased in rat dams that are diabetic for 1 week prior to pregnancy (increase in placental lipoprotein lipase), however, the expression of *Igf2* and the IGF signalling machinery is decreased and the abundance of *GLUT1/Slc2a1* reduced in association with a more minor increase in fetal weight (by 5%) (Cisse *et al.*, 2013). Genetically-inducing maternal insulin insensitivity by a global heterozygous deficiency in PI3K-p110α signalling capacity in the mouse dam is associated with improved placental Lz development (larger surface area and thinner barrier to diffusion), but reduced glucose transport and expression of nutrient (*GLUT1/Slc2a1, SNAT1/Slc38a1, SNAT2/Slc38a2*) and prolactin-related family genes near term (Sferruzzi-Perri *et al.*, 2016). However, the specific nature of these placental changes depended on whether the conceptus itself was heterozygous for the PI3K-p110α deficiency (Sferruzzi-Perri *et al.*, 2016). Moreover, there is no effect of maternal heterozygous deficiency in PI3K-p110α signalling on fetal weight in this model, irrespective of fetal genotype (Sferruzzi-Perri *et al.*, 2016). Taken together, these studies suggest that the IGF2/PI3K-p110α system plays an important role in modulating fetal nutrition and growth in response to maternal insulin deficiency and/or insensitivity, by acting at the level of placental transport phenotype.

Other environmental challenges affecting conceptus growth

The expression of *Igf*s, receptors and signalling machinery in the placenta also changes in response insults that affect the placental capacity to supply the fetus with nutrients. Such insults include reduced utero-placental blood flow, heat stress and alcohol consumption (Table 3). Reducing both

maternal oxygen and nutrient supply to the conceptus using uterine artery ligation in mice, rats and guinea pigs, or placental embolism in sheep, reduces placental expression of components of the IGF system in association with defects in placental Lz structure and in transporter expression and activity of the glucose and Systems A and L amino acid transporters (Table 3). The extent of these changes however, depends on timing of the insult in the pregnancy. In sheep, removal of uterine caruncles prior to pregnancy is associated with increased placental *Igf2* expression and an adaptive increase in placentome size, trophoblast and maternal capillary volume and surface area, although total placental mass and fetal weight, are reduced [Table 3; (Zhang *et al.*, 2016b)]. Acute exogenous IGF1 does not alter nutrient metabolism by the embolised sheep placenta [Table 1; (Jensen *et al.*, 1999)]. However, several doses of intra-amniotic IGF1 increases glucose and Systems A and L amino acid transporter expression by the embolised placenta and improves feto-placental growth *in vivo* [Table 1; (Eremia *et al.*, 2007; Wali *et al.*, 2012)]. Moreover, in mice with uterine artery ligation, targeting of IGF1 to the placenta using a nanoparticle or adenoviral-mediated approach increases the abundance of glucose and Systems A and L amino acid transporters in the placenta, placental width and fetal growth [Table 1; (Jones *et al.*, 2013; Jones *et al.*, 2014; Abd Ellah *et al.*, 2015)]. These findings highlight the therapeutic potential of IGFs for improving the capacity of the placenta to supply nutrients to the fetus in compromised pregnancies.

In ewes, heat stress reduces placental growth and glucose transport capacity, as well as, alters the expression of IGF1 and IGF2, and AKT, mTORC1 and MAPK signalling pathways during gestation (Table 3). In rats, alcohol consumption during the peri-conceptional period leads to late gestational fetal growth restriction but no change in placental weight (Gardebjer *et al.*, 2014). However, Lz development and *Igf1*, *Igf1r* and *SNAT2/Slc38a2* expression is decreased, but Jz glycogen cell formation, *Igf2* and GLUT1/Slc2a1 may be increased in response to peri-conceptional alcohol exposure near term (Gardebjer *et al.*, 2014). This suggests there can be programmed changes in the conceptus leading to changes in the IGF system and the structural and functional phenotype of the placenta that link the maternal environment from the earliest stages of pregnancy to fetal growth near term.

CONCLUSIONS AND PERSPECTIVES

Thus, IGFs are important regulators of placental resource allocation to fetal growth both developmentally and in response to environmental manipulations known to program the ill health of offspring. They increase placental morphogenesis, substrate transport and hormone secretion,

599 which, in turn promotes fetal growth either directly via the supply of nutrients and oxygen or
600 indirectly via the maternal metabolic adaptation to pregnancy and the availability of nutrients for
601 transplacental transport. In response to environmental challenges, the IGFs (particularly IGF2) and
602 their signalling pathways change in line with the alterations in placental structure and function, and
603 thereby, link changes in the maternal environment to fetal substrate supply and growth during
604 pregnancy with implications for developmental programming. The environmentally-induced changes
605 in the IGF system and placental phenotype may be beneficial (obesogenic diets, moderate hypoxia)
606 or detrimental (eg. severe oxygen and nutrient deprivation and glucocorticoid excess) to resource
607 allocation to the fetus depending on the type, severity and timing of the challenge during pregnancy
608 (Figure 3). The beneficial effects of IGF treatments on placental phenotype show promising
609 therapeutic potential for improving fetal growth in situations in which placental growth is impaired
610 without major maternal compromise, particularly when the treatment with IGF1 or IGF2 is targeted
611 directly to the placenta. However, efforts to understand the regulation of endogenous placental IGF
612 expression may also be fruitful, particularly in the case of *Igf2* which appears to be most important
613 for mediating adaptive responses locally in mice. These findings are important in the context of
614 human pregnancy as dysregulated expression of the IGFs and signalling components are often
615 reported in the human placenta associated with abnormal fetal growth (Abu-Amero *et al.*, 1998;
616 Sheikh *et al.*, 2001; Gratton *et al.*, 2002; Gurel *et al.*, 2003; Laviola *et al.*, 2005; Scioscia *et al.*, 2006;
617 Street *et al.*, 2006; Trollmann *et al.*, 2007; Akram *et al.*, 2008; Yung *et al.*, 2008; Colomiere *et al.*,
618 2009; Borzsonyi *et al.*, 2011; Street *et al.*, 2011; Demendi *et al.*, 2012; Jansson *et al.*, 2012a; Iniguez
619 *et al.*, 2014; Nawathe *et al.*, 2016; Zhang *et al.*, 2016a). However, it is important to note, that several
620 causes of environmental, maternal, and fetal origin, can lead to changes in placental phenotype and
621 fetal growth in humans (Gaccioli & Lager, 2016). Thus studies of animal models showing alterations
622 in the expression of IGFs and their signalling pathways provides insight but further information is
623 required on the natural conditions of variable placental phenotype among humans.
624
625
626 **REFERENCES**

627 Abd Ellah N, Taylor L, Troja W, Owens K, Ayres N, Pauletti G & Jones H. (2015). Development of non-
628 viral, trophoblast-specific gene delivery for placental therapy. *PLoS One* **10,** e0140879.
629
630 Abu-Amero SN, Ali Z, Bennett P, Vaughan JI & Moore GE. (1998). Expression of the insulin-like
631 growth factors and their receptors in term placentas: a comparison between normal and
632 IUGR births. *Molecular Reproduction and Development* **49,** 229-235.
633

634 Ain R, Canham LN & Soares MJ. (2005). Dexamethasone-induced intrauterine growth restriction
635 impacts the placental prolactin family, insulin-like growth factor-II and the Akt signaling
636 pathway. *Journal of Endocrinology* **185,** 253-263.

637
638 Akram SK, Akram M, Bhutta ZA & Soder O. (2008). Human placental IGF-I and IGF-II expression:
639 correlating maternal and infant anthropometric variables and micronutrients at birth in the
640 Pakistani population. *Acta Paediatrica* **97,** 1443-1448.

641
642 Anderson AH, Fennessey PV, Meschia G, Wilkening RB & Battaglia FC. (1997). Placental transport of
643 threonine and its utilization in the normal and growth-restricted fetus. *The American Journal*
644 *of Physiology* **272,** E892-900.

645
646 Angiolini E, Coan PM, Sandovici I, Iwajomo OH, Peck G, Burton GJ, Sibley CP, Reik W, Fowden AL &
647 Constancia M. (2011). Developmental adaptations to increased fetal nutrient demand in
648 mouse genetic models of Igf2-mediated overgrowth. *FASEB* **25,** 1737-1745.

649
650 Arroyo JA, Anthony RV, Parker TA & Galan HL. (2010). eNOS, NO, and the activation of ERK and AKT
651 signaling at mid-gestation and near-term in an ovine model of intrauterine growth
652 restriction. *Systems Biology in Reprodroductive Medicine* **56,** 62-73.

653
654 Arroyo JA, Brown LD & Galan HL. (2009). Placental mammalian target of rapamycin and related
655 signaling pathways in an ovine model of intrauterine growth restriction. *American Journal of*
656 *Obstetrics and Gynecology* **201,** 616 e611-617.

657
658 Aye IL, Rosario FJ, Powell TL & Jansson T. (2015). Adiponectin supplementation in pregnant mice
659 prevents the adverse effects of maternal obesity on placental function and fetal growth.
660 *Proc Natl Acad Sci U S A* **112,** 12858-12863.

661
662 Bach LA. (2015). Insulin-Like Growth Factor Binding Proteins--an Update. *Pediatric Endocrinology*
663 *Reviews* **13,** 521-530.

664
665 Bach LA, Headey SJ & Norton RS. (2005). IGF-binding proteins--the pieces are falling into place.
666 *Trends in Endocrinology and Metabolism* **16,** 228-234.

667
668 Baker J, Liu JP, Robertson EJ & Efstratiadis A. (1993). Role of insulin-like growth factors in embryonic
669 and postnatal growth. *Cell* **75,** 73-82.

670
671 Baumann MU, Schneider H, Malek A, Palta V, Surbek DV, Sager R, Zamudio S & Illsley NP. (2014).
672 Regulation of human trophoblast GLUT1 glucose transporter by insulin-like growth factor I
673 (IGF-I). *PLoS One* **9,** e106037.

674
675 Bhaumick B, George D & Bala RM. (1992). Potentiation of epidermal growth factor-induced
676 differentiation of cultured human placental cells by insulin-like growth factor-I. *The Journal*
677 *of Clinical Endocrinology and Metabolism* **74,** 1005-1011.

678
679 Bloomfield FH, Bauer MK, van Zijl PL, Gluckman PD & Harding JE. (2002a). Amniotic IGF-I
680 supplements improve gut growth but reduce circulating IGF-I in growth-restricted fetal
681 sheep. *American Journal of Physiology, Endocrinology and Metabolism* **282,** E259-269.
682
683 Bloomfield FH, van Zijl PL, Bauer MK & Harding JE. (2002b). A chronic low dose infusion of insulin-like
684 growth factor I alters placental function but does not affect fetal growth. *Reprodroduction,*
685 *Fertility and Develpoment* **14,** 393-400.
686
687 Bloxam DL, Bax BE & Bax CM. (1994). Epidermal growth factor and insulin-like growth factor I
688 differently influence the directional accumulation and transfer of 2-aminoisobutyrate (AIB)
689 by human placental trophoblast in two-sided culture. *Biochemical and Biophysical Research*
690 *Communications* **199,** 922-929.
691
692 Borzsonyi B, Demendi C, Nagy Z, Toth K, Csanad M, Pajor A, Rig J, Jr. & Joo JG. (2011). Gene
693 expression patterns of insulin-like growth factor 1, insulin-like growth factor 2 and insulin-
694 like growth factor binding protein 3 in human placenta from pregnancies with intrauterine
695 growth restriction. *Journal of Perinatal Medicine* **39,** 701-707.
696
697 Brett KE, Ferraro ZM, Holcik M & Adamo KB. (2015). Prenatal physical activity and diet composition
698 affect the expression of nutrient transporters and mTOR signaling molecules in the human
699 placenta. *Placenta* **36,** 204-212.
700
701 Brett KE, Ferraro ZM, Holcik M & Adamo KB. (2016). Placenta nutrient transport-related gene
702 expression: the impact of maternal obesity and excessive gestational weight gain. The
703 *Journal of Maternal-Fetal and Neonatal Medicine* **29,** 1399-1405.
704
705 Burton GJ, Fowden AL & Thornburg KL. (2016). Placental Origins of Chronic Disease. *Physiological*
706 *Reviews* **96,** 1509-1565.
707
708 Carter AM. (2007). Animal Models of Human Placentation - A Review. *Placenta* **28,** S41-S47.
709
710 Carter AM, Kingston MJ, Han KK, Mazzuca DM, Nygard K & Han VK. (2005). Altered expression of
711 IGFs and IGF-binding proteins during intrauterine growth restriction in guinea pigs. *Journal*
712 *of Endocrinology* **184,** 179-189.
713
714 Charnock JC, Dilworth MR, Aplin JD, Sibley CP, Westwood M & Crocker IP. (2016). The impact of a
715 human IGF-II analog ([Leu27]IGF-II) on fetal growth in a mouse model of fetal growth
716 restriction. *American Journal of Physiology, Endocrinology and Metabolism* **310,** E24-31.
717
718 Cho H, Thorvaldsen JL, Chu Q, Feng F & Birnbaum MJ. (2001). Akt1/PKBalpha is required for normal
719 growth but dispensable for maintenance of glucose homeostasis in mice. *Journal of*
720 *Biological Chemistry* **276,** 38349-38352.
721

722 Church DN, Phillips BR, Stuckey DJ, Barnes DJ, Buffa FM, Manek S, Clarke K, Harris AL, Carter EJ &
723 Hassan AB. (2012). Igf2 ligand dependency of Pten(+/-) developmental and tumour
724 phenotypes in the mouse. *Oncogene* **31,** 3635-3646.

725
726 Cisse O, Fajardy I, Dickes-Coopman A, Moitrot E, Montel V, Deloof S, Rousseaux J, Vieau D & Laborie
727 C. (2013). Mild gestational hyperglycemia in rat induces fetal overgrowth and modulates
728 placental growth factors and nutrient transporters expression. *PLoS One* **8,** e64251.

729
730 Clemmons DR. (2016). Role of IGF Binding Proteins in Regulating Metabolism. *Trends in*
731 *Endocrinology and Metabolism* **27,** 375-391.

732
733 Coan PM, Angiolini E, Sandovici I, Burton GJ, Constancia M & Fowden AL. (2008a). Adaptations in
734 placental nutrient transfer capacity to meet fetal growth demands depend on placental size
735 in mice. *Journal of Physiology* **586,** 4567-4576.

736
737 Coan PM, Fowden AL, Constancia M, Ferguson-Smith AC, Burton GJ & Sibley CP. (2008b).
738 Disproportional effects of Igf2 knockout on placental morphology and diffusional exchange
739 characteristics in the mouse. *Journal of Physiology* **586,** 5023-5032.

740
741 Coan PM, Vaughan OR, McCarthy J, Mactier C, Burton GJ, Constancia M & Fowden AL. (2011).
742 Dietary composition programmes placental phenotype in mice. *Journal of Physiology*.

743
744 Coan PM, Vaughan OR, Sekita Y, Finn SL, Constancia M, Burton GJ & Fowden AL. (2010). Adaptations
745 in placental phenotype support fetal growth during undernutrition of pregnant mice. *Journal*
746 *of Physiology* **588,** 527-538.

747
748 Cohran V, Fang J, Milio L, Smith CH & Fant M. (1996). Type I insulin-like growth factor receptors in
749 the BeWo choriocarcinoma cell (b30 clone) during cell differentiation. *Placenta* **17,** 313-320.

750
751 Colomiere M, Permezel M, Riley C, Desoye G & Lappas M. (2009). Defective insulin signaling in
752 placenta from pregnancies complicated by gestational diabetes mellitus. *European Journal of*
753 *Endocrinology* **160,** 567-578.

754
755 Constancia M, Angiolini E, Sandovici I, Smith P, Smith R, Kelsey G, Dean W, Ferguson-Smith A, Sibley
756 CP, Reik W & Fowden A. (2005). Adaptation of nutrient supply to fetal demand in the mouse
757 involves interaction between the Igf2 gene and placental transporter systems. *Proc Natl*
758 *Acad Sci USA* **102,** 19219-19224.

759
760 Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, Stewart F, Kelsey G,
761 Fowden A, Sibley C & Reik W. (2002). Placental-specific IGF-II is a major modulator of
762 placental and fetal growth. *Nature* **417,** 945-948.

763
764 Corcoran JJ, Charnock JC, Martin J, Taggart MJ & Westwood M. (2012). Differential effect of insulin
765 like growth factor-I on constriction of human uterine and placental arteries. *The Journal of*
766 *Clinical Endocrinology and Metabolism* **97,** E2098-2104.

Cuffe JS, Dickinson H, Simmons DG & Moritz KM. (2011). Sex specific changes in placental growth and MAPK following short term maternal dexamethasone exposure in the mouse. *Placenta* **32,** 981-989.

Cuffe JS, Walton SL, Singh RR, Spiers JG, Bielefeldt-Ohmann H, Wilkinson L, Little MH & Moritz KM. (2014). Mid- to late term hypoxia in the mouse alters placental morphology, glucocorticoid regulatory pathways and nutrient transporters in a sex-specific manner. *Journal of Physiology* **592,** 3127-3141.

Das UG, Sadiq HF, Soares MJ, Hay WW, Jr. & Devaskar SU. (1998). Time-dependent physiological regulation of rodent and ovine placental glucose transporter (GLUT-1) protein. *The American Journal of Physiology* **274,** R339-347.

de Vrijer B, Davidsen ML, Wilkening RB, Anthony RV & Regnault TR. (2006). Altered placental and fetal expression of IGFs and IGF-binding proteins associated with intrauterine growth restriction in fetal sheep during early and mid-pregnancy. *Pediatric Research* **60,** 507-512.

de Vrijer B, Regnault TR, Wilkening RB, Meschia G & Battaglia FC. (2004). Placental uptake and transport of ACP, a neutral nonmetabolizable amino acid, in an ovine model of fetal growth restriction. *American Journal of Physiology, Endocrinology and Metabolism* **287,** E1114-1124.

DeChiara TM, Efstratiadis A & Robertson EJ. (1990). A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345,** 78-80.

DeChiara TM, Robertson EJ & Efstratiadis A. (1991). Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64,** 849-859.

Demendi C, Borzsonyi B, Nagy ZB, Rigo J, Jr., Pajor A & Joo JG. (2012). Gene expression patterns of insulin-like growth factor 1, 2 (IGF-1, IGF-2) and insulin-like growth factor binding protein 3 (IGFBP-3) in human placenta from preterm deliveries: influence of additional factors. *European Journal of Obstetrics and Gynecology and Reprodroductive Biology* **160,** 40-44.

Diaz LE, Chuan Y-C, Lewitt M, Fernandez-Perez L, Carrasco-Rodriguez S, Sanchez-Gomez M & Flores-Morales A. (2007). IGF-II regulates metastatic properties of choriocarcinoma cells through the activation of the insulin receptor. *Molecular Human Reprodroduction* **13,** 567-576.

Diaz P, Harris J, Rosario FJ, Powell TL & Jansson T. (2015). Increased placental fatty acid transporter 6 and binding protein 3 expression and fetal liver lipid accumulation in a mouse model of obesity in pregnancy. *American Journal of Physiology, Regulatory Integrative and Comparative Physiology* **309,** R1569-1577.

Dilworth MR, Andersson I, Renshall LJ, Cowley E, Baker P, Greenwood S, Sibley CP & Wareing M. (2013). Sildenafil citrate increases fetal weight in a mouse model of fetal growth restriction with a normal vascular phenotype. *PLoS One* **8,** e77748.

Dilworth MR, Kusinski LC, Cowley E, Ward BS, Husain SM, ConstÃ¢ncia M, Sibley CP & Glazier JD. (2010). Placental-specific Igf2 knockout mice exhibit hypocalcemia and adaptive changes in placental calcium transport. *Proc Natl Acad Sci USA* **107,** 3894-3899.

Dong Y, Zhang L, Zhang S, Bai Y, Chen H, Sun X, Yong W, Li W, Colvin SC, Rhodes SJ, Shou W & Zhang ZY. (2012). Phosphatase of regenerating liver 2 (PRL2) is essential for placental development by down-regulating PTEN (Phosphatase and Tensin Homologue Deleted on Chromosome 10) and activating Akt protein. *Journal of Biological Chemistry* **287,** 32172-32179.

Eckert JJ, Porter R, Watkins AJ, Burt E, Brooks S, Leese HJ, Humpherson PG, Cameron IT & Fleming TP. (2012). Metabolic induction and early responses of mouse blastocyst developmental programming following maternal low protein diet affecting life-long health. *PLoS One* **7,** e52791.

Eremia SC, de Boo HA, Bloomfield FH, Oliver MH & Harding JE. (2007). Fetal and Amniotic Insulin-Like Growth Factor-I Supplements Improve Growth Rate in Intrauterine Growth Restriction Fetal Sheep. *Endocrinology* **148,** 2963-2972.

Esquiliano DR, Guo W, Liang L, Dikkes P & Lopez MF. (2009). Placental glycogen stores are increased in mice with H19 null mutations but not in those with insulin or IGF type 1 receptor mutations. *Placenta* **30,** 693-699.

Fang J, Mao D, Smith CH & Fant ME. (2006). IGF regulation of neutral amino acid transport in the BeWo choriocarcinoma cell line (b30 clone): evidence for MAP kinase-dependent and MAP kinase-independent mechanisms. *Growth Hormone IGF Research* **16,** 318-325.

Feldser D, Agani F, Iyer NV, Pak B, Ferreira G & Semenza GL. (1999). Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Research* **59,** 3915-3918.

Fernandez AM & Torres-Aleman I. (2012). The many faces of insulin-like peptide signalling in the brain. *Nature Reviews Neuroscience* **13,** 225-239.

Forbes K, Shah VK, Siddals K, Gibson JM, Aplin JD & Westwood M. (2015). Statins inhibit insulin-like growth factor action in first trimester placenta by altering insulin-like growth factor 1 receptor glycosylation. *Molecular Human Reprodion* **21,** 105-114.

Forbes K, West G, Garside R, Aplin JD & Westwood M. (2009). The protein-tyrosine phosphatase, Src homology-2 domain containing protein tyrosine phosphatase-2, is a crucial mediator of exogenous insulin-like growth factor signaling to human trophoblast. *Endocrinology* **150,** 4744-4754.

Forbes K, Westwood M, Baker PN & Aplin JD. (2008). Insulin-like growth factor I and II regulate the life cycle of trophoblast in the developing human placenta. *Americal Journal Physiology Celularl Physiology* **294,** C1313-1322.

Fowden AL. (2003). The insulin-like growth factors and feto-placental growth. *Placenta* **24,** 803-812.

Fowden AL, Giussani DA & Forhead AJ. (2006). Intrauterine programming of physiological systems: causes and consequences. *Physiology (Bethesda)* **21,** 29-37.

Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M & Burton GJ. (2009). Placental efficiency and adaptation: endocrine regulation. *Journal of Physiology* **587,** 3459-3472.

Gabory A, Ferry L, Fajardy I, Jouneau L, Gothie JD, Vige A, Fleur C, Mayeur S, Gallou-Kabani C, Gross MS, Attig L, Vambergue A, Lesage J, Reusens B, Vieau D, Remacle C, Jais JP & Junien C. (2012). Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS One* **7,** e47986.

Gaccioli F & Lager S. (2016). Placental Nutrient Transport and Intrauterine Growth Restriction. *Frontiers in Physiology* **7,** 40.

Gaccioli F, White V, Capobianco E, Powell TL, Jawerbaum A & Jansson T. (2013). Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2alpha signaling and increases fetal growth in rats. *Biology of Reproduction* **89,** 96.

Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vige A, Breton C, Reusens B, Remacle C, Vieau D, Ekstrom TJ, Jais JP & Junien C. (2010). Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. *PLoS One* **5,** e14398.

Gao H, Sathishkumar KR, Yallampalli U, Balakrishnan M, Li X, Wu G & Yallampalli C. (2012a). Maternal protein restriction regulates IGF2 system in placental labyrinth. *Frontiers in Bioscience* **4,** 1434-1450.

Gao H, Yallampalli U & Yallampalli C. (2012b). Gestational protein restriction reduces expression of Hsd17b2 in rat placental labyrinth. *Biology of Reproduction* **87,** 68.

Gao H, Yallampalli U & Yallampalli C. (2013). Gestational protein restriction affects trophoblast differentiation. *Frontiers in Bioscience* **5,** 591-601.

Gardebjer EM, Cuffe JS, Pantaleon M, Wlodek ME & Moritz KM. (2014). Periconceptional alcohol consumption causes fetal growth restriction and increases glycogen accumulation in the late gestation rat placenta. *Placenta* **35,** 50-57.

Gardner RL, Squire S, Zaina S, Hills S & Graham CF. (1999). Insulin-like growth factor-2 regulation of conceptus composition: effects of the trophectoderm and inner cell mass genotypes in the mouse. *Biology of Reproduction* **60,** 190-195.

902 Gluckman PD, Hanson MA, Morton SM & Pinal CS. (2005). Life-long echoes--a critical analysis of the
903 developmental origins of adult disease model. *Biology f the Neonate* **87,** 127-139.

904
905 Gratton RJ, Asano H & Han VK. (2002). The regional expression of insulin-like growth factor II (IGF-II)
906 and insulin-like growth factor binding protein-1 (IGFBP-1) in the placentae of women with
907 pre-eclampsia. *Placenta* **23,** 303-310.

908
909 Gurel D, Ozer E, Altunyurt S, Guclu S & Demir N. (2003). Expression of IGR-IR and VEGF and
910 trophoblastic proliferative activity in placentas from pregnancies complicated by IUGR.
911 *Pathology, Research and Practice* **199,** 803-809.

912
913 Habli M, Jones H, Aronow B, Omar K & Crombleholme TM. (2013). Recapitulation of characteristics
914 of human placental vascular insufficiency in a novel mouse model. *Placenta* **34,** 1150-1158.

915
916 Hamilton GS, Lysiak JJ, Han VK & Lala PK. (1998). Autocrine-paracrine regulation of human
917 trophoblast invasiveness by insulin-like growth factor (IGF)-II and IGF-binding protein
918 (IGFBP)-1. *Experimental Cell Research* **244,** 147-156.

919
920 Harding JE, Liu L, Evans PC & Gluckman PD. (1994). Insulin-like growth factor 1 alters feto-placental
921 protein and carbohydrate metabolism in fetal sheep. *Endocrinology* **134,** 1509-1514.

922
923 Harris LK, Crocker IP, Baker PN, Aplin JD & Westwood M. (2011). IGF2 actions on trophoblast in
924 human placenta are regulated by the insulin-like growth factor 2 receptor, which can
925 function as both a signaling and clearance receptor. *Biology of Reproduction* **84,** 440-446.

926
927 Harris LK & Westwood M. (2012). Biology and significance of signalling pathways activated by IGF-II.
928 *Growth Factors* **30,** 1-12.

929
930 Hashimoto R, Sakai K, Matsumoto H & Iwashita M. (2010). Tumor necrosis factor-alpha (TNF-alpha)
931 inhibits insulin-like growth factor-I (IGF-I) activities in human trophoblast cell cultures
932 through IGF-I/insulin hybrid receptors. *Endocrine Journal* **57,** 193-200.

933
934 Hatano N, Mori Y, Oh-hora M, Kosugi A, Fujikawa T, Nakai N, Niwa H, Miyazaki J, Hamaoka T & Ogata
935 M. (2003). Essential role for ERK2 mitogen-activated protein kinase in placental
936 development. *Genes Cells* **8,** 847-856.

937
938 Hauguel-de Mouzon S, Louizeau M & Girard J. (1992). Functional alterations of type I insulin-like
939 growth factor receptor in placenta of diabetic rats. *The Biochemical Journal* **288,** 273-279.

940
941 He B, Zhang N, Jia Y, Sun Q & Zhao R. (2016). Glucocorticoid receptor-mediated insulin-like growth
942 factor-I transcriptional regulation in BeWo trophoblast cells before and after syncytialisation.
943 *Steroids* **115,** 26-33.

944

945 Higgins JS, Vaughan OR, de Liger EF, Fowden AL & Sferruzzi-Perri AN. (2015). Placental phenotype
946 and resource allocation to fetal growth are modified by the timing and degree of hypoxia
947 during mouse pregnancy. *Journal of Physiology* **594,** 1341-1356.

948
949 Hills FA, Mehmet H & Sullivan MH. (2012). Insulin-like growth factor-II and heparin are anti-
950 apoptotic survival factors in human villous cytotrophoblast. *European Journal of Obstetrics*
951 *and Gynecology and Reprodroductive Biology* **163,** 11-16.

952
953 Hunt LC, Xu B, Finkelstein D, Fan Y, Carroll PA, Cheng PF, Eisenman RN & Demontis F. (2015). The
954 glucose-sensing transcription factor MLX promotes myogenesis via myokine signaling. *Genes*
955 *& Development* **29,** 2475-2489.

956
957 Iniguez G, Castro JJ, Garcia M, Kakarieka E, Johnson MC, Cassorla F & Mericq V. (2014). IGF-IR signal
958 transduction protein content and its activation by IGF-I in human placentas: relationship
959 with gestational age and birth weight. *PLoS One* **9,** e102252.

960
961 Irving JA & Lala PK. (1995). Functional role of cell surface integrins on human trophoblast cell
962 migration: regulation by TGF-beta, IGF-II, and IGFBP-1. *Experimental Cell Research* **217,** 419-
963 427.

964
965 Jansson N, Pettersson J, Haafiz A, Ericsson A, Palmberg I, Tranberg M, Ganapathy V, Powell TL &
966 Jansson T. (2006). Down-regulation of placental transport of amino acids precede the
967 development of intrauterine growth restriction in rats fed a low protein diet. *Journal of*
968 *Physiology*.

969
970 Jansson N, Rosario FJ, Gaccioli F, Lager S, Jones HN, Roos S, Jansson T & Powell TL. (2012a).
971 Activation of placental mTOR signaling and amino acid transporters in obese women giving
972 birth to large babies. *The Journal of Clinical Endocrinology and Metabolism* **98,** 105-113.

973
974 Jansson T, Aye IL & Goberdhan DC. (2012b). The emerging role of mTORC1 signaling in placental
975 nutrient-sensing. *Placenta* **33,** e23-29.

976
977 Jansson T & Persson E. (1990). Placental transfer of glucose and amino acids in intrauterine growth
978 retardation: studies with substrate analogs in the awake guinea pig. *Pediatric Research* **28,**
979 203-208.

980
981 Jansson T & Powell TL. (2006). IFPA 2005 Award in Placentology Lecture. Human placental transport
982 in altered fetal growth: does the placenta function as a nutrient sensor? -- a review. *Placenta*
983 **27,** S91-97.

984
985 Jensen EC, Harding JE, Bauer MK & Gluckman PD. (1999). Metabolic effects of IGF-I in the growth
986 retarded fetal sheep. *Journal of Endocrinology* **161,** 485-494.

987
988 Jensen EC, van Zijl P, Evans PC & Harding JE. (2000). Effect of IGF-I on serine metabolism in fetal
989 sheep. *Journal of Endocrinology* **165,** 261-269.

Jeong W, Song G, Bazer FW & Kim J. (2014). Insulin-like growth factor I induces proliferation and migration of porcine trophectoderm cells through multiple cell signaling pathways, including protooncogenic protein kinase 1 and mitogen-activated protein kinase. *Molecular Cellular Endocrinology* **384,** 175-184.

Jones H, Crombleholme T & Habli M. (2014). Regulation of amino acid transporters by adenoviral-mediated human insulin-like growth factor-1 in a mouse model of placental insufficiency in vivo and the human trophoblast line BeWo in vitro. *Placenta* **35,** 132-138.

Jones HN, Crombleholme T & Habli M. (2013). Adenoviral-mediated placental gene transfer of IGF-1 corrects placental insufficiency via enhanced placental glucose transport mechanisms. *PLoS One* **8,** e74632.

Kanai-Azuma M, Kanai Y, Kurohmaru M, Sakai S & Hayashi Y. (1993). Insulin-like growth factor (IGF)-I stimulates proliferation and migration of mouse ectoplacental cone cells, while IGF-II transforms them into trophoblastic giant cells in vitro. *Biology of Reproduction* **48,** 252-261.

Karl PI. (1995). Insulin-like growth factor-1 stimulates amino acid uptake by the cultured human placental trophoblast. *J Cell Physiol* **165,** 83-88.

Katz AB, Keswani SG, Habli M, Lim FY, Zoltick PW, Midrio P, Kozin ED, Herlyn M & Crombleholme TM. (2009). Placental gene transfer: transgene screening in mice for trophic effects on the placenta. *American Journal of Obstetrics and Gynecology* **201,** 499 e491-498.

Kavitha JV, Rosario FJ, Nijland MJ, McDonald TJ, Wu G, Kanai Y, Powell TL, Nathanielsz PW & Jansson T. (2014). Down-regulation of placental mTOR, insulin/IGF-I signaling, and nutrient transporters in response to maternal nutrient restriction in the baboon. *FASEB M***28,** 1294-1305.

Keesey RE & Hirvonen MD. (1997). Body weight set-points: determination and adjustment. *Journal of Nutrition* **127,** 1875S-1883S.

Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G & Reik W. (2012). The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nature Cell Biology* **14,** 659-665.

Kent LN, Ohboshi S & Soares MJ. (2012). Akt1 and insulin-like growth factor 2 (Igf2) regulate placentation and fetal/postnatal development. *International Journal of Developmental Biology* **56,** 255-261.

Keswani SG, Balaji S, Katz AB, King A, Omar K, Habli M, Klanke C & Crombleholme TM. (2015). Intraplacental gene therapy with Ad-IGF-1 corrects naturally occurring rabbit model of intrauterine growth restriction. *Human Gene Therapy* **26,** 172-182.

Kim J, Song G, Gao H, Farmer JL, Satterfield MC, Burghardt RC, Wu G, Johnson GA, Spencer TE & Bazer FW. (2008). Insulin-like growth factor II activates phosphatidylinositol 3-kinase-protooncogenic protein kinase 1 and mitogen-activated protein kinase cell signaling pathways, and stimulates migration of ovine trophectoderm cells. *Endocrinology* **149,** 3085-3094.

King A, Ndifon C, Lui S, Widdows K, Kotamraju VR, Agemy L, Teesalu T, Glazier JD, Cellesi F, Tirelli N, Aplin JD, Ruoslahti E & Harris LK. (2016). Tumor-homing peptides as tools for targeted delivery of payloads to the placenta. *Science Advances* **2,** e1600349.

King V, Hibbert N, Seckl JR, Norman JE & Drake AJ. (2013). The effects of an obesogenic diet during pregnancy on fetal growth and placental gene expression are gestation dependent. *Placenta* **34,** 1087-1090.

Kniss DA, Shubert PJ, Zimmerman PD, Landon MB & Gabbe SG. (1994). Insulin like growth factors. Their regulation of glucose and amino acid transport in placental trophoblasts isolated from first-trimester chorionic villi. *Journal of Reproductive Medicine* **39,** 249-256.

Kusinski LC, Dilworth MR, Baker PN, Sibley CP, Wareing M & Glazier JD. (2011). System A activity and vascular function in the placental-specific Igf2 knockout mouse. *Placenta* **32,** 871-876.

Kwong WY, Miller DJ, Ursell E, Wild AE, Wilkins AP, Osmond C, Anthony FW & Fleming TP. (2006). Imprinted gene expression in the rat embryo-fetal axis is altered in response to periconceptional maternal low protein diet. *Reproduction Supplement* **132,** 265-277.

Lacey H, Haigh T, Westwood M & Aplin JD. (2002). Mesenchymally-derived Insulin-like growth factor provides a paracrine stimulus for trophoblast migration. *BMC Developmental Biology* **2,** 5.

Lager S, Samulesson AM, Taylor PD, Poston L, Powell TL & Jansson T. (2014). Diet-induced obesity in mice reduces placental efficiency and inhibits placental mTOR signaling. *Physiological Reports* **2,** e00242.

Laviola L, Perrini S, Belsanti G, Natalicchio A, Montrone C, Leonardini A, Vimercati A, Scioscia M, Selvaggi L, Giorgino R, Greco P & Giorgino F. (2005). Intrauterine growth restriction in humans is associated with abnormalities in placental insulin-like growth factor signaling. *Endocrinology* **146,** 1498-1505.

Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A & Tilghman SM. (1995). Disruption of imprinting caused by deletion of the H19 gene region in mice. *Nature* **375,** 34-39.

Li C, Levitz M, Hubbard GB, Jenkins SL, Han V, Ferry Jr. RJ, McDonald TJ, Nathanielsz PW & Schlabritz-Loutsevitch NE. (2007). The IGF axis in baboon pregnancy: Placental and systemic responses to feeding 70% global ad libitum diet. *Placenta* **28,** 1200-1210.

Liu JP, Baker J, Perkins AS, Robertson EJ & Efstratiadis A. (1993). Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* **75,** 59-72.

Liu L, Harding JE, Evans PC & Gluckman PD. (1994). Maternal insulin-like growth factor-I infusion alters feto-placental carbohydrate and protein metabolism in pregnant sheep. *Endocrinology* **135,** 895-900.

Lopez MF, Dikkes P, Zurakowski D & Villa-Komaroff L. (1996). Insulin-like growth factor II affects the appearance and glycogen content of glycogen cells in the murine placenta. *Endocrinology* **137,** 2100-2108.

Louvi A, Accili D & Efstratiadis A. (1997). Growth-promoting interaction of IGF-II with the insulin receptor during mouse embryonic development. *Developmental Biology* **189,** 33-48.

Ludwig T, Eggenschwiler J, Fisher P, D'Ercole AJ, Davenport ML & Efstratiadis A. (1996). Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in Igf2 and Igf1r null backgrounds. *Developmental Biology* **177,** 517-535.

Ma Y, Zhu MJ, Uthlaut AB, Nijland MJ, Nathanielsz PW, Hess BW & Ford SP. (2011). Upregulation of growth signaling and nutrient transporters in cotyledons of early to mid-gestational nutrient restricted ewes. *Placenta* **32,** 255-263.

Ma Y, Zhu MJ, Zhang L, Hein SM, Nathanielsz PW & Ford SP. (2010). Maternal obesity and overnutrition alter fetal growth rate and cotyledonary vascularity and angiogenic factor expression in the ewe. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **299,** R249-258.

Magnusson-Olsson AL, Hamark B, Ericsson A, Wennergren M, Jansson T & Powell T. (2006). Gestational and hormonal regulation of human placental lipoprotein lipase. *Journal Lipid Research* **47,** 2551-61.

Mark PJ, Sisala C, Connor K, Patel R, Lewis JL, Vickers M, Waddell BJ & Sloboda DM. (2011). A maternal high-fat diet in rat pregnancy reduces growth of the fetus and the placental junctional zone, but not placental labyrinth zone growth. *Journal of Developmental Origins of Health and Disease* **2,** 63-70.

Martinez N, Capobianco E, White V, Pustovrh MC, Higa R & Jawerbaum A. (2008). Peroxisome proliferator-activated receptor alpha activation regulates lipid metabolism in the feto-placental unit from diabetic rats. *Reproduction Supplement* **136,** 95-103.

Martino J, Sebert S, Segura MT, Garcia-Valdes L, Florido J, Padilla MC, Marcos A, Rueda R, McArdle HJ, Budge H, Symonds ME & Campoy C. (2016). Maternal body weight and gestational diabetes differentially influence placental and pregnancy outcomes. *The Journal of Clinical Endocrinology and Metabolism* **101,** 59-68.

Maruo T, Murata K, Matsuo H, Samoto T & Mochizuki M. (1995). Insulin-like growth factor-I as a local regulator of proliferation and differentiated function of the human trophoblast in early pregnancy. *Early Pregnancy* **1,** 54-61.

Matheson H, Veerbeek JH, Charnock-Jones DS, Burton GJ & Yung HW. (2015). Morphological and molecular changes in the murine placenta exposed to normobaric hypoxia throughout pregnancy. *Journal of Physiology* **594,** 1371-1388

Matthews JC, Beveridge MJ, Dialynas E, Bartke A, Kilberg MS & Novak DA. (1999). Placental anionic and cationic amino acid transporter expression in growth hormone overexpressing and null IGF-II or null IGF-I receptor mice. *Placenta* **20,** 639-650.

Mayama R, Izawa T, Sakai K, Suciu N & Iwashita M. (2013). Improvement of insulin sensitivity promotes extravillous trophoblast cell migration stimulated by insulin-like growth factor-I. *Endocrine Journal* **60,** 359-368.

McKinnon T, Chakraborty C, Gleeson LM, Chidiac P & Lala PK. (2001). Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. *The Journal of Clinical Endocrinology and Metabolism* **86,** 3665-3674.

McMillen IC & Robinson JS. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiological Reviews* **85,** 571-633.

McMullen S, Osgerby JC, Milne JS, Wallace JM & Wathes DC. (2005). The effects of acute nutrient restriction in the mid-gestational ewe on maternal and fetal nutrient status, the expression of placental growth factors and fetal growth. *Placenta* **26,** 25-33.

Milio LA, Hu J & Douglas GC. (1994). Binding of insulin-like growth factor I to human trophoblast cells during differentiation in vitro. *Placenta* **15,** 641-651.

Miller AG, Aplin JD & Westwood M. (2005). Adenovirally mediated expression of insulin-like growth factors enhances the function of first trimester placental fibroblasts. *The Journal of Clinical Endocrinology and Metabolism* **90,** 379-385.

Mina TH, Raikkonen K, Riley SC, Norman JE & Reynolds RM. (2015). Maternal distress associates with placental genes regulating fetal glucocorticoid exposure and IGF2: Role of obesity and sex. *Psychoneuroendocrinology* **59,** 112-122.

Monk D, Arnaud P, Apostolidou S, Hills FA, Kelsey G, Stanier P, Feil R & Moore GE. (2006). Limited evolutionary conservation of imprinting in the human placenta. *Proc Natl Acad Sci U S A* **103,** 6623-6628.

Moore T, Constancia M, Zubair M, Bailleul B, Feil R, Sasaki H & Reik W. (1997). Multiple imprinted sense and antisense transcripts, differential methylation and tandem repeats in a putative imprinting control region upstream of mouse Igf2. *Proc Natl Acad Sci U S A* **94,** 12509-12514.

Nawathe AR, Christian M, Kim SH, Johnson M, Savvidou MD & Terzidou V. (2016). Insulin-like growth factor axis in pregnancies affected by fetal growth disorders. *Clinical Epigenetics* **8,** 11.

Nusken KD, Schneider H, Plank C, Trollmann R, Nusken E, Rascher W & Dotsch J. (2011). Fetal programming of gene expression in growth-restricted rats depends on the cause of low birth weight. *Endocrinology* **152,** 1327-1335.

Okamoto T, Katada T, Murayama Y, Ui M, Ogata E & Nishimoto I. (1990). A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. *Cell* **62,** 709-717.

Olausson H & Sohlstrom A. (2003). Effects of food restriction and pregnancy on the expression of insulin-like growth factors-I and -II in tissues from guinea pigs. *Journal of Endocrinology* **179,** 437-445.

Osgerby JC, Gadd TS & Wathes DC. (2003). Effect of maternal body condition on placental and fetal growth and the insulin-like growth factor axis in Dorset ewes. *Reproduction Supplement* **125,** 717-731.

Osgerby JC, Wathes DC, Howard D & Gadd TS. (2002). The effect of maternal undernutrition on ovine fetal growth. *Journal of Endocrinology* **173,** 131-141.

Osgerby JC, Wathes DC, Howard D & Gadd TS. (2004). The effect of maternal undernutrition on the placental growth trajectory and the uterine insulin-like growth factor axis in the pregnant ewe. *Journal of Endocrinology* **182,** 89-103.

Pankevich DE, Mueller BR, Brockel B & Bale TL. (2009). Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. *Physiology Behavior* **98,** 94-102.

Pantham P, Rosario FJ, Nijland M, Cheung A, Nathanielsz PW, Powell TL, Galan HL, Li C & Jansson T. (2015). Reduced placental amino acid transport in response to maternal nutrient restriction in the baboon. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **309,** R740-746.

Pantham P, Rosario FJ, Weintraub ST, Nathanielsz PW, Powell TL, Li C & Jansson T. (2016). Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in maternal nutrient restricted baboons. *Biology of Reproduction* **95,** 98.

Petry CJ, Evans ML, Wingate DL, Ong KK, Reik W, ConstÃ¢ncia M & Dunger DB. (2010). Raised late pregnancy glucose concentrations in mice carrying pups withtargeted disruption of H19Delta13. *Diabetes* **59,** 282-286.

Pollheimer J, Haslinger P, Fock V, Prast J, Saleh L, Biadasiewicz K, Jetne-Edelmann R, Haraldsen G, Haider S, Hirtenlehner-Ferber K & Knofler M. (2011). Endostatin suppresses IGF-II-mediated signaling and invasion of human extravillous trophoblasts. *Endocrinology* **152,** 4431-4442.

Price WA, Rong L, Stiles AD & D'Ercole AJ. (1992). Changes in IGF-I and -II, IGF binding protein, and IGF receptor transcript abundance after uterine artery ligation. *Pediatric Research* **32,** 291-295.

Pringle KG, Kind KL, Thompson JG & Roberts CT. (2007). Complex interactions between hypoxia inducible factors, insulin-like growth factor-II and oxygen in early murine trophoblasts. *Placenta* **28,** 1147-1157.

Qiu Q, Basak A, Mbikay M, Tsang BK & Gruslin A. (2005). Role of pro-IGF-II processing by proprotein convertase 4 in human placental development. *Proc Natl Acad Sci U S A* **102,** 11047-11052.

Rak-Mardyla A & Gregoraszczuk EL. (2010). Effect of pituitary growth hormone and insulin-like growth factor type-I on proliferation, apoptosis and hormone secretion of the placental cell line JEG-3. *Gynecological Endocrinology* **26,** 302-306.

Regnault TR, de Vrijer B, Galan HL, Wilkening RB, Battaglia FC & Meschia G. (2007). Development and mechanisms of fetal hypoxia in severe fetal growth restriction. *Placenta* **28,** 714-723.

Regnault TR, Marconi AM, Smith CH, Glazier JD, Novak DA, Sibley CP & Jansson T. (2005). Placental amino acid transport systems and fetal growth restriction--a workshop report. *Placenta* **26,** S76-80.

Regnault TRH, Vrijer Bd, Galan HL, Davidsen ML, Trembler KA, Battaglia FC, Wilkening RB & Anthony RV. (2003). The relationship between transplacental O2 diffusion and placental expression of PlGF, VEGF and their receptors in a placental insufficiency model of fetal growth restriction *Journal of Physiology* **550,** 641-656.

Reid GJ, Flozak AS & Simmons RA. (2002). Placental expression of insulin-like growth factor receptor-1 and insulin receptor in the growth-restricted fetal rat. *Journal of the Society for Gynecologic Investigation* **9,** 210-214.

Ripoche MA, Kress C, Poirier F & Dandolo L. (1997). Deletion of the H19 transcription unit reveals the existence of a putative imprinting control element. *Genes & development* **11,** 1596-1604.

Roberts CT, Sohlstrom A, Kind KL, Earl RA, Khong TY, Robinson JS, Owens PC & Owens JA. (2001). Maternal food restriction reduces the exchange surface area and increases the barrier thickness of the placenta in the guinea-pig. *Placenta* **22,** 177-185.

Roberts RM, Green JA & Schulz LC. (2016). The evolution of the placenta. *Reproduction Supplment* **152,** R179-189.

Rosario FJ, Jansson N, Kanai Y, Prasad PD, Powell TL & Jansson T. (2011). Maternal Protein Restriction in the Rat Inhibits Placental Insulin, mTOR, and STAT3 Signaling and Down-Regulates Placental Amino Acid Transporters. *Endocrinology* **152,** 1119-1129.

Rosario FJ, Kanai Y, Powell TL & Jansson T. (2015). Increased placental nutrient transport in a novel mouse model of maternal obesity with fetal overgrowth. *Obesity* **23,** 1663-1670.

Rosario FJ, Powell TL & Jansson T. (2016). Activation of placental insulin and mTOR signaling in a mouse model of maternal obesity associated with fetal overgrowth. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **310,** R87-93.

Ross JC, Fennessey PV, Wilkening RB, Battaglia FC & Meschia G. (1996). Placental transport and fetal utilization of leucine in a model of fetal growth retardation. *The American Journal of Physiology* **270,** E491-503.

Sandovici I, Hoelle K, Angiolini E & Constancia M. (2012). Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. *Reproductive Biomedicine Online* **25,** 68-89.

Sasson IE, Vitins AP, Mainigi MA, Moley KH & Simmons RA. (2015). Pre-gestational vs gestational exposure to maternal obesity differentially programs the offspring in mice. *Diabetologia* **58,** 615-624.

Scioscia M, Gumaa K, Kunjara S, Paine MA, Selvaggi LE, Rodeck CH & Rademacher TW. (2006). Insulin resistance in human preeclamptic placenta is mediated by serine phosphorylation of insulin receptor substrate-1 and -2. *The Journal of Clinical Endocrinology and Metabolism* **91,** 709-717.

Sferruzzi-Perri AN & Camm EJ. (2016). The programming power of the placenta. *Frontiers in Physiology* **7,**33.

Sferruzzi-Perri AN, Lopez-Tello J, Fowden AL & Constancia M. (2016). Maternal and fetal genomes interplay through phosphoinositol 3-kinase(PI3K)-p110α signalling to modify placental resource allocation. *Proc Natl Acad Sci USA* **113,** 11255-11260.

Sferruzzi-Perri AN, Owens JA, Pringle KG & Roberts CT. (2010). The neglected role of insulin-like growth factors in the maternal circulation regulating fetal growth. *Journal of Physiology* **589,** 7-20.

Sferruzzi-Perri AN, Owens JA, Pringle KG, Robinson JS & Roberts CT. (2006). Maternal insulin-like growth factors-I and -II act via different pathways to promote fetal growth. *Endocrinology* **147,** 3344-3355.

Sferruzzi-Perri AN, Owens JA, Standen P & Roberts CT. (2008). Maternal insulin-like growth factor-II promotes placental functional development via the type 2 IGF receptor in the guinea pig. *Placenta* **29,** 347-355.

Sferruzzi-Perri AN, Owens JA, Standen P, Taylor RL, Heinemann GK, Robinson JS & Roberts CT. (2007a). Early treatment of the pregnant guinea pig with IGFs promotes placental transport and nutrient partitioning near term. *American Journal of Physiology, Endocrinology and Metabolism* **292,** E668-676.

Sferruzzi-Perri AN, Owens JA, Standen P, Taylor RL, Robinson JS & Roberts CT. (2007b). Early pregnancy maternal endocrine IGF-I programs the placenta for increased functional capacity throughout gestation. *Endocrinology* **148,** 4362-4370.

Sferruzzi-Perri AN, Vaughan OR, Coan PM, Suciu MC, Darbyshire R, Constancia M, Burton GJ & Fowden AL. (2011). Placental-specific Igf2 deficiency alters developmental adaptations to undernutrition in mice. *Endocrinology* **152,** 3202-3212.

Sferruzzi-Perri AN, Vaughan OR, Haro M, Cooper WN, Musial B, Charalambous M, Pestana D, Ayyar S, Ferguson-Smith AC, Burton GJ, Constancia M & Fowden AL. (2013). An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB* **27,** 3928-3937.

Shaikh S, Bloomfield FH, Bauer MK, Phua HH, Gilmour RS & Harding JE. (2005). Amniotic IGF-I supplementation of growth-restricted fetal sheep alters IGF-I and IGF receptor type 1 mRNA and protein levels in placental and fetal tissues. *Journal of Endocrinology* **186,** 145-155.

Sheikh S, Satoskar P & Bhartiya D. (2001). Expression of insulin-like growth factor-I and placental growth hormone mRNA in placentae: a comparison between normal and intrauterine growth retardation pregnancies. *Molecular Human Reproduction* **7,** 287-292.

Shields SK, Nicola C & Chakraborty C. (2007). Rho guanosine 5'-triphosphatases differentially regulate insulin-like growth factor I (IGF-I) receptor-dependent and -independent actions of IGF-II on human trophoblast migration. *Endocrinology* **148,** 4906-4917.

Sibley CP, Coan PM, Ferguson-Smith AC, Dean W, Hughes J, Smith P, Reik W, Burton GJ, Fowden AL & Constancia M. (2004). Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *Proc Natl Acad Sci USA* **101,** 8204-8208.

Siler-Khodr TM, Forman J & Sorem KA. (1995). Dose-related effect of IGF-I on placental prostanoid release. *Prostaglandins* **49,** 1-14.

Skeffington KL, Higgins JS, Mahmoud AD, Evans AM, Sferruzzi-Perri AN, Fowden AL, Yung HW, Burton GJ, Giussani DA & Moore LG. (2015). Hypoxia, AMPK activation and uterine artery vasoreactivity. *Journal of Physiology* **594,** 1357-1369.

Sohlstrom A, Fernberg P, Owens JA & Owens PC. (2001). Maternal nutrition affects the ability of treatment with IGF-I and IGF-II to increase growth of the placenta and fetus, in guinea pigs. *Growth Hormone IGF Research* **11,** 392-398.

Standen P, Sferruzzi-Perri AN, Taylor R, Heinemann G, Zhang JV, Highet AR, Pringle KG, Owens JA, Kumarasamy V, Lumbers ER & Roberts CT. (2015). Maternal insulin-like growth factor 1 and 2 differentially affect the renin-angiotensin system during pregnancy in the guinea pig. *Growth Hormone IGF Research* **25,** 141-147.

Street ME, Seghini P, Fieni S, Ziveri MA, Volta C, Martorana D, Viani I, Gramellini D & Bernasconi S. (2006). Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns with fetal growth restriction compared with controls. *European Journal of Endocrinology* **155,** 567-574.

Street ME, Viani I, Ziveri MA, Volta C, Smerieri A & Bernasconi S. (2011). Impairment of insulin receptor signal transduction in placentas of intra-uterine growth-restricted newborns and its relationship with fetal growth. *European Journal of Endocrinology* **164,** 45-52.

Thureen PJ, Trembler KA, Meschia G, Makowski EL & Wilkening RB. (1992). Placental glucose transport in heat-induced fetal growth retardation. *The American Journal of Physiology* **263,** R578-585.

Trollmann R, Klingmuller K, Schild RL, Rascher W & Dotsch J. (2007). Differential gene expression of somatotrophic and growth factors in response to in vivo hypoxia in human placenta. *American Journal of Obstetrics and Gynecology* **197,** 601 e601-606.

Tuersunjiang N, Odhiambo JF, Long NM, Shasa DR, Nathanielsz PW & Ford SP. (2013). Diet reduction to requirements in obese/overfed ewes from early gestation prevents glucose/insulin dysregulation and returns fetal adiposity and organ development to control levels. *American Journal of Physiology, Endocrinology and Metabolism* **305,** E868-878.

Van Mieghem T, van Bree R, Van Herck E, Deprest J & Verhaeghe J. (2009). Insulin-like growth factor-II regulates maternal hemodynamic adaptation to pregnancy in rats. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **297,** R1615-1621.

Van Winkle LJ, Tesch JK, Shah A & Campione AL. (2006). System B0,+ amino acid transport regulates the penetration stage of blastocyst implantation with possible long-term developmental consequences through adulthood. *Human Reproduction Update* **12,** 145-157..

Vaughan OR, Fisher HM, Dionelis KN, Jefferies EC, Higgins JS, Musial B, Sferruzzi-Perri AN & Fowden AL. (2015). Corticosterone alters materno-fetal glucose partitioning and insulin signalling in pregnant mice. *Journal of Physiology* **593,** 1307-1321.

Vaughan OR, Forhead AJ & Fowden AL. (2011). Glucocorticoids and placental programming. In *The Placenta and Human Developmental Programming*, ed. Burton GJ, Barker DJP & Moffett A, pp. 175-187. Cambridge University Press, Cambridge.

Vaughan OR, Sferruzzi-Perri AN & Fowden AL. (2012). Maternal corticosterone regulates nutrient allocation tofetal growth in mice. *Journal of Physiology* **590,** 5529-5540.

Wali JA, de Boo HA, Derraik JG, Phua HH, Oliver MH, Bloomfield FH & Harding JE. (2012). Weekly intra-amniotic IGF-1 treatment increases growth of growth-restricted ovine fetuses and up-regulates placental amino acid transporters. *PLoS One* **7,** e37899.

Watkins AJ, Lucas ES, Marfy-Smith S, Bates N, Kimber SJ & Fleming TP. (2015). Maternal nutrition modifies trophoblast giant cell phenotype and fetal growth in mice. *Reproduction Supplement* **149,** 563-575.

White V, Jawerbaum A, Mazzucco MB, Gauster M, Desoye G & Hiden U. (2015). Diabetes-associated changes in the fetal insulin/insulin-like growth factor system are organ specific in rats. *Pediatric Research* **77,** 48-55.

Wlodek ME, Westcott KT, OÂ'Dowd R, Serruto A, Wassef L, Moritz KM & Moseley JM. (2005). Uteroplacental restriction in the rat impairs fetal growth in association with alterations in placental growth factors including PTHrP. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **288,** R1620-R1627.

Wooding FB & Burton GJ. (2008). *Comparative Placentation; Structures, Functions and Evolution*. Springer, Berlin.

Yang Z-Z, Tschopp O, Hemmings-Mieszczak M, Feng J, Brodbeck D, Perentes E & Hemmings BA. (2003). Protein kinase B{alpha}/Akt1 regulates placental development and fetal growth. *Journal of Biological Chemistry* **278,** 32124-32131.

Youssef A & Han VK. (2016). Low Oxygen Tension Modulates the Insulin-Like Growth Factor-1 or -2 Signaling via Both Insulin-Like Growth Factor-1 Receptor and Insulin Receptor to Maintain Stem Cell Identity in Placental Mesenchymal Stem Cells. *Endocrinology* **157,** 1163-1174.

Youssef A, Iosef C & Han VK. (2014). Low-oxygen tension and IGF-I promote proliferation and multipotency of placental mesenchymal stem cells (PMSCs) from different gestations via distinct signaling pathways. *Endocrinology* **155,** 1386-1397.

Yu J, Iwashita M, Kudo Y & Takeda Y. (1998). Phosphorylated insulin-like growth factor (IGF)-binding protein-1 (IGFBP-1) inhibits while non-phosphorylated IGFBP-1 stimulates IGF-I-induced amino acid uptake by cultured trophoblast cells. *Growth Hormone IGF Res* **8,** 65-70.

1434 Yung H-w, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS & Burton GJ. (2008).
1435 Evidence of Placental Translation Inhibition and Endoplasmic Reticulum Stress in the Etiology
1436 of Human Intrauterine Growth Restriction. *American Journal of Pathology* **173,** 451-462.

1437
1438 Yung HW, Cox M, Tissot van Patot M & Burton GJ. (2012a). Evidence of endoplasmic reticulum stress
1439 and protein synthesis inhibition in the placenta of non-native women at high altitude. *FASEB*
1440 **26,** 1970-1981.

1441
1442 Yung HW, Hemberger M, Watson ED, Senner CE, Jones CP, Kaufman RJ, Charnock-Jones DS & Burton
1443 GJ. (2012b). Endoplasmic reticulum stress disrupts placental morphogenesis: implications for
1444 human intrauterine growth restriction. *The Journal of Pathology* **228,** 554-564.

1445
1446 Zamudio S, Baumann MU & Illsley NP. (2006). Effects of chronic hypoxia in vivo on the expression of
1447 human placental glucose transporters. *Placenta* **27,** 49-55.

1448
1449 Zeck W, Widberg C, Maylin E, Desoye G, Lang U, McIntyre D, Prins J & Russell A. (2008). Regulation of
1450 placental growth hormone secretion in a human trophoblast model--the effects of
1451 hormones and adipokines. *Pediatric Research* **63,** 353-357.

1452
1453 Zhang B, Jin Z, Sun L, Zheng Y, Jiang J, Feng C & Wang Y. (2016a). Expression and correlation of sex
1454 hormone-binding globulin and insulin signal transduction and glucose transporter proteins in
1455 gestational diabetes mellitus placental tissue. *Diabetes Research and Clinical Practice* **119,**
1456 106-117.

1457
1458 Zhang S, Barker P, Botting KJ, Roberts CT, McMillan CM, McMillen IC & Morrison JL. (2016b). Early
1459 restriction of placental growth results in placental structural and gene expression changes in
1460 late gestation independent of fetal hypoxemia. *Physiological Reports* **4,** e13049.

1461
1462 Zhu MJ, Du M, Hess BW, Means WJ, Nathanielsz PW & Ford SP. (2007a). Maternal Nutrient
1463 Restriction Upregulates Growth Signaling Pathways in the Cotyledonary Artery of Cow
1464 Placentomes. *Placenta* **28,** 361-368.

1465
1466 Zhu MJ, Du M, Hess BW, Nathanielsz PW & Ford SP. (2007b). Periconceptional nutrient restriction in
1467 the ewe alters MAPK/ERK1/2 and PI3K/Akt Growth signaling pathways and vascularity in the
1468 placentome. *Placenta* **28,** 1192-1199.

1469
1470 Zhu MJ, Du M, Nijland MJ, Nathanielsz PW, Hess BW, Moss GE & Ford SP. (2009). Down-regulation of
1471 growth signaling pathways linked to a reduced cotyledonary vascularity in placentomes of
1472 over-nourished, obese pregnant ewes. *Placenta* **30,** 405-410.

1473
1474 Zhu MJ, Ma Y, Long NM, Du M & Ford SP. (2010). Maternal obesity markedly increases placental
1475 fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe.
1476 *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **299,**
1477 R1224-R1231.

1478

FUNDING

ANS-P is funded by a Royal Society Dorothy Hodgkin Research Fellowship.

ACKNOWLEDGEMENTS

We thank Dr Fatima Santos for helping us prepare the Figures.

Abstract figure. The proposed actions of IGFs on placental resource allocation to drive fetal growth. Note that changes in placental IGFs and resource allocation depend on the timing and severity of the environmental insult.

Figure 1. Impact of exogenous IGFs on the placenta. A) The effect of exogenous IGFs on placental human trophoblast *in vitro.* Proposed signalling pathways mediating the actions of IGFs shown. **B)** The effect of exogenous maternal IGFs on the mouse, rat and/or guinea pig placenta *in vivo*. Dashed lines indicate a potential interaction (A) or impact (B) of IGF1. IGF = insulin-like growth factor, IGF1R = type 1 IGF receptor, IGF2R = type 2 IGF receptor, INSR = insulin receptor, Jz = junctional zone, Lz = labyrinthine zone, MAPK = mitogen-activated protein kinase, PI3K = phosphoinositol 3-kinase.

Figure 2. The effect of genetically manipulating IGF2 expression or signalling on placental phenotype in mice. A) shows the effect of complete loss of IGF2 and **B)** shows the effect of partial loss of IGF2, either by deleting the placental-exclusive isoform, *Igf2P0* or through a constitutive heterozygous deficiency of PI3K-p110α. Dashed line indicates a potential interaction of IGF2 with receptor. Line with a round head indicates parameters reduced by loss of IGF2 signalling. Loss of IGF2 signalling leads to reductions in placental development and transport function (A). Partial loss of IGF2 signalling also leads to reductions in placental development, but is associated with adaptive up-regulation in transport function (B). AA = amino acids, IGF = insulin-like growth factor, IGF1R = type 1 IGF receptor, Lz = labyrinthine zone, MAPK = mitogen-activated protein kinase, PI3K = phosphoinositol 3-kinase, XRp = unknown placental-specific IGF receptor.

Figure 3. The effect of different environmental manipulations on the placental IGF system and resource allocation phenotype in the mouse. A) shows manipulations which down-regulate IGF2

signalling. **B)** shows manipulations which up-regulate IGF2 signalling. AKT = protein kinase B, IGF = insulin-like growth factor, Lz = labyrinthine zone. * Note *Igf2P0* is required for the placenta to up-regulate amino acid transport to the fetus in response to maternal undernutrition.

1532 **Table 1**. The impact of exogenous IGF1 or IGF2 on the placental phenotype and fetal outcome (where available)

IGF	System	Species	Treatment	Study	Placental size and morphology	Placental function	Fetal weight	References
IGF1	*In vitro*	Mouse	Primary ectoplacental cone trophoblast	First trimester	↑ proliferation and migration			(Kanai-Azuma *et al.*, 1993)
		Pig	Primary trophoblast cells	First trimester	↑ proliferation and migration			(Jeong *et al.*, 2014)
		Human	1st trimester primary trophoblast	First trimester	↑ invasion via INSR and IGF1R activation of Akt			(Mayama *et al.*, 2013)
		Human	1st trimester placental explant	First trimester	↑ proliferation and syncytial formation via IGF1R-mediated MAPK signalling,↓ apoptosis via IGF1R-mediated PI3K signalling			(Forbes *et al.*, 2008; Forbes *et al.*, 2015)
		Human	1st trimester placental trophoblast	First trimester	↑ proliferation, migration			(Hashimoto *et al.*, 2010)
		Human	1st trimester placental explant	First trimester	↑ proliferation			(Forbes *et al.*, 2009; Forbes *et al.*, 2015)
		Human	1st trimester placental explant	First trimester	↑ migration			(Lacey *et al.*, 2002)

		Human	1st trimester placental explant	First trimester	↑ proliferation	↑hCG, hPL		(Maruo *et al.*, 1995)
		Human	1st trimester trophoblast	First trimester		↑System A amino acid and glucose uptake		(Kniss *et al.*, 1994)
		Human	1st trimester primary placental fibroblasts	First trimester	↑proliferation, invasion, ↓ apoptosis			(Miller *et al.*, 2005) *Ad-IGF-I
		Human	BeWo syncytial cell line		↑ proliferation, invasion, ↓ apoptosis	↑ System A and System L amino acid transporter activity, Snat1, Snat2, Lat1, 4F2hc, GLUT1, GLUT3 and GLUT8, ↓Lat2		(Jones *et al.*, 2013; Jones *et al.*, 2014) *Ad-hIGF-I
		Human	BeWo			↔ pGH		(Zeck *et al.*, 2008)
		Human	JEG-3 choriocarcinoma cell line		↑proliferation, ↓apoptosis	↑ P4, hCG secretion		(Rak-Mardyla & Gregoraszczuk, 2010)
		Human	JEG-3		↑invasion via induction of adhesion and migration through IGF1R-PI3K and MAPK signalling			(Diaz *et al.*, 2007)
		Human	BeWo			↑ System A amino acid transporter activity via PI3K signalling, ↔ Snat1 or		(Fang *et al.*, 2006)

						Snat2		
		Human	BeWo, term explants and term perfused human placenta			↑ glucose transport, GLUT1 membrane abundance		(Baumann *et al.*, 2014)
		human	Term human placenta	Term		↓ LPL activity in		(Magnusson-Olsson *et al.*, 2006)
		Human	Term trophoblast	Term		↑System A amino acid uptake		(Bloxam *et al.*, 1994; Karl, 1995; Yu *et al.*, 1998)
		Human	Term trophoblast and cell lines	Term	↑ syncytialisation			(Bhaumick *et al.*, 1992; Milio *et al.*, 1994; Cohran *et al.*, 1996)
IGF2	*In vitro*	Mouse	Primary ectoplacental cone trophoblast	First trimester	↑ differentiation into endocrine cells			(Kanai-Azuma *et al.*, 1993)
		Sheep	Primary trophoblast	First trimester	↑ migration			(Kim *et al.*, 2008)
		Human	1st trimester HTR8_SVneo cell line	First trimester	↑ migration via Rho GTPases			(Qiu *et al.*, 2005; Shields *et al.*, 2007)
		Human	1st trimester HTR8_SVneo cell line	First trimester	↑ migration via signalling through IGF2R involving inhibitory G proteins and the MAPK pathway			(McKinnon *et al.*, 2001)

		Human	1st trimester primary trophoblast	First trimester	↑ migration/invasion			(Irving & Lala, 1995; Hamilton *et al.*, 1998)
		Human	1st trimester placental explant	First trimester	↑ trophoblast proliferation and syncytial formation via IGF1R-mediated MAPK signalling, ↓ apoptosis via IGF1R-mediated PI3K signalling			(Forbes *et al.*, 2008; Forbes *et al.*, 2009; Forbes *et al.*, 2015)
		Human	JEG-3 choriocarcinoma cell line		↑ invasion via induction of adhesion and migration through INSR-PI3K and MAPK signalling			(Diaz *et al.*, 2007)
		Human	SGHPL4 and 1st trimester villous explants	First trimester	↑ proliferation, migration and invasion			(Pollheimer *et al.*, 2011)
		Human	1st trimester primary placental fibroblasts	First trimester	↑ proliferation and invasion, ↓ apoptosis			(Miller *et al.*, 2005) *Ad-IGF-II
		Human	1st trimester and term trophoblast	First trimester	↓ apoptosis, ↑proliferation and survival against TNF-α and IFN-γ-induced apoptosis			(Hills *et al.*, 2012)
		Human	1st trimester placental	First		↑ glucose uptake		(Kniss *et al.*, 1994)

			trophoblast	trimester				
		Human	1st trimester placental trophoblast	First trimester		↑ glucose and System A amino acid uptake		(Kniss *et al.*, 1994)
		Human	1st trimester placental explant	First trimester	↑ migration			(Lacey *et al.*, 2002)
		Human	In BeWo and term explants		↑ proliferation ↓ apoptosis and necrosis			(Harris *et al.*, 2011)
IGF1	*In vivo*	Mouse	D14	D17	↔ weight, ↑ placental cross-sectional area, Lz and fetal and maternal facing areas		↔ weight or viability	(Katz *et al.*, 2009) * Ad-hIGF-I
		Mouse uterine artery ligation	D16	D20	↔ weight, ↑placental thickness		↑27%, ↔ fetal viability	(Abd Ellah *et al.*, 2015) * nanoparticle targeted delivery to placenta: PLAC1-hIGF-1
		Mouse uterine artery ligation	D18	D20	ND	↑4F2hc, Lat1, Lat2, GLUT8, GLUT9a/b, ↔ Snat1, Snat2,	ND	(Jones *et al.*, 2013; Jones *et al.*, 2014)

						GLUT1		* Ad-hIGF-I
		Guinea pig	D20-37	D40	↔ weight		↑ 6%, ↓ litter size	(Sohlstrom *et al.*, 2001)
		Guinea pig	D20-38	D35	↑ 17% weight, ↓ placental and Lz area, ↔ Lz, Jz, FC, MBS, Troph Vd	↑ glucose and System A amino acid transfer, Snat2 and prorenin activation, ↓ Igf2, ↔ Glut1, Igf1	↑ 15%	(Sferruzzi-Perri *et al.*, 2007b; Standen *et al.*, 2015)
		Guinea pig	D20-38	D62	↔ weight ↔ structure	↑ glucose and System A amino acid transfer	↑ 17% ↑ fetal viability	(Sferruzzi-Perri *et al.*, 2006; Sferruzzi-Perri *et al.*, 2007a)
		Guinea pig 30%UN	D20-37	D40	↑ 13% weight		↔	(Sohlstrom *et al.*, 2001)
		Rabbit Natural runt in litter	D19	D21	↔ weight		↑ 19%	(Keswani *et al.*, 2015) * Ad-hIGF-I

		Sheep	D128, 4hr infusion	D128	ND	↑ glucose transfer and lactate production, ↔ blood flow, urea or glucose transfer	ND	(Liu *et al.*, 1994)
		Sheep * Fetal infusion	D121-132	D132	↔ weight, ↓ placentome number	↓ glucose and System A amino acid transfer	↔	(Bloomfield *et al.*, 2002b)
		Sheep * Fetal infusion	D128, 4hr infusion	D128	ND	↓ glucose transfer, lactate uptake and umbilical flow, ↔ urea transfer or serine uptake	ND	(Harding *et al.*, 1994; Jensen *et al.*, 1999; Jensen *et al.*, 2000)
		Sheep Embolised * Fetal infusion	D128, 4hr infusion	D128	ND	↔ glucose or urea transfer, lactate uptake and umbilical flow	ND	(Jensen *et al.*, 1999)
		Sheep Spontaneous growth restriction * Fetal infusion	D128, 4hr infusion	D128	ND	↔ glucose or urea transfer, lactate uptake and umbilical flow	ND	(Jensen *et al.*, 1999)
		Sheep Embolised * Intra-	D110, D117, D124	D120-131	↔ but placentas no longer significantly different to untreated controls	↔ glucose uptake, ↑ Glut1, Glut4, Systems y+ and L transporters (Slc7a1 and	↔ weight, ↑ fetal growth rate and fetuses no longer significantly different	(Eremia *et al.*, 2007; Wali *et al.*, 2012)

		amniotic infusion				Slc7a8) ↔ Glut3, Snat4, Slc7a5	to untreated controls	
IGF2	*In vivo*	Mouse	D14, D16, D18 IGF2 (1mg/kg/day) or iRGD-liposome with IGF2 (0.3mg/kg/day)	D18	↑ weight		↔	(King *et al.*, 2016)
		Mouse IGF2P0	D14, D16, D18 treatment with iRGD-liposome with IGF2 (0.3mg/kg/day)	D18	↔ weight of Igf2P0 and WT		↑ Igf2P0 but not WT	(King *et al.*, 2016)
		Rat	D16-22	D22	↔ weight, ↑ Jz		↔	(Van Mieghem *et al.*, 2009)
		Guinea pig	D20-37	D40	↑ 9% weight		↑ 7%	(Sohlstrom *et al.*, 2001)
		Guinea pig	D20-38	D35	↔ weight and structure	↔ glucose or System A amino acid transfer, Glut1, Snat2, Igf1 and Igf2,↑prorenin activation	↔	(Sferruzzi-Perri *et al.*, 2007b; Standen *et al.*, 2015)
		Guinea pig	D20-38	D62	↔ weight, ↑ Lz area, Vd, Vol, SA, ↓ Jz Vd, ↔ BT	↑ glucose transfer, ↔ System A amino acid transfer	↑ 11% weight and ↑ fetal viability	(Sferruzzi-Perri *et al.*, 2006; Sferruzzi-Perri *et al.*, 2007a)

		Guinea pig 30%UN	D20-37	D40	↔ weight		↔	(Sohlstrom *et al.*, 2001)
Leu^{-27} IGF-II	*In vivo*	Mouse	D13-19	D19	↔ weight	↔ System A amino acid transfer, ↓ litter System A amino acid variability	↔ weight, ↓ variability in fetal weight	(Charnock *et al.*, 2016)
		Mouse eNOS-/-	D13-19	D19	↔ weight		↑	(Charnock *et al.*, 2016)
		Guinea pig	D20-38	D62	↔ weight, ↑ Lz vd, Troph, MBS Vd and Vol and SA, ↓ Jz area, Vd, Vol, FC Vd, Vol and BT	↑ glucose and System A transfer and prorenin activation	↑ 11%	(Sferruzzi-Perri *et al.*, 2008)

For *in vivo* studies, exogenous IGF was administered to the mother, unless stated otherwise. Abbreviations: BT=barrier thickness, D=day, FC=fetal capillaries, GLUT=glucose transporter, hCG=human chorionic gonadotrophin, hPL=human placental lactogen, IGF1/Igf1=insulin-like growth factor-1, IGF2/Igf2=insulin-like growth factor-2, Jz=junctional zone, LAT=cationic amino acid transporter, Lz=labyrinthine zone, MAPK/ERK=mitogen activated kinase, MBS=maternal blood space, ND=not determined; P4=progesterone; PI3K=phosphoinositol 3-kinase, pGH=placental growth hormone, Prl=prolactin-related hormone, SA=surface area, SNAT/Slc38a= Sodium-coupled neutral amino acid transporter, vol=volume, vd=volume density.

Search terms used: trophoblast, placenta, fetus, insulin-like growth factor, IGF and/or transport

1539 **Table 2. The effect of genetically manipulating IGF abundance and/or signalling on feto-placental growth in mice**

Manipulation	Approach	Placental			Fetal weight	Reference
		size	morphology	function		
Deficiency of IGF and downstream signalling						
Global IGF1 KO	Igf1-/-	D18/19 ↔			D18/19 ↓40%	(Baker *et al.*, 1993)
Global IGF2 KO	Paternal Igf2-	D15 ↓47% D18/19 ↓20-30%	D15 ↓ Jz GlyT D18/19 ↔ Lz and Jz Vd ↓ Jz GlyT	D17 ↓ EAAT1, EAAT2 (Jz), EAAT3 (Jz), EAAT4,↑ CAT1, ↔ 4f2hc D18/19 ND	D15 ND D18/19 ↓40% ↑ fetal loss	(DeChiara *et al.*, 1990; DeChiara *et al.*, 1991; Baker *et al.*, 1993; Liu *et al.*, 1993; Lopez *et al.*, 1996; Matthews *et al.*, 1999; Esquiliano *et al.*, 2009; Church *et al.*, 2012; Kent *et al.*, 2012)
Global IGF2 KO	Paternal transmission LacZDMR2–	D16 ↓27% D19 ↓40%	D16 ND D19 ↓ Lz vd and volume of all Lz components, SA, FC length and diffusing capacity, ↑ Jz vd and BT	D16 ↔ System A and glucose transport, Snat1, Snat2, Snat4 D19 ↓ System A transfer and passive permeability and Snat2, ↔ glucose transport, Snat1 and Snat4	D16 ↓24% D19 ↓52%	(Constancia *et al.*, 2005; Coan *et al.*, 2008b)
Fetal specific IGF2 KO	Inner cell mass Igf2-	D17 ↓14%			D17 ↓27%	(Gardner *et al.*, 1999)
Placental trophoblast specific IGF2 KO	Trophechoderm Igf2-	D17 ↓21%			D17 ↓12%	(Gardner *et al.*, 1999)
Placental Lz specific	Paternal transmission	D16 ↓20%	D16 ↔ Lz or Jz Vd	D16 ↑ System A and	D16 ↔/↓4%	(Constancia *et al.*, 2002;

IGF2 KO	Igf2P0-	D17 ↓24% D19 ↓35%	↓ Lz Trophoblast, GlyT D19 ↓ SA, trophoblast, FC volume, FC length, diffusing capacity, ↑ BT ↔ Lz or Jz vd and umbilical artery flow	glucose transport, Snat4, Glut3, ↓ passive permeability, calbindin, ↔ Snat1, Snat2, Glut1 D19 ↑/↔ System A transport, ↑glucose and calcium transport, ↓ passive permeability, ↔ Snat1, Snat2, Snat4, calcium transport, calbindin, PMCA1, TRPV6	D17 ↓24% D19 ↓24%	Sibley *et al.*, 2004; Constancia *et al.*, 2005; Coan *et al.*, 2008b; Dilworth *et al.*, 2010; Kusinski *et al.*, 2011; Sferruzzi-Perri *et al.*, 2011; Dilworth *et al.*, 2013)
Global IGF1R KO	Igf1r-	D19 ↔	D18/19 ↔ Jz GlyT	D17 ↓ EAAT2 (Jz), EAAT3 (Lz and Jz), ↑ CAT1, ↔ EAAT1,EAAT4	D19 ↓55%	(DeChiara *et al.*, 1990; Louvi *et al.*, 1997; Matthews *et al.*, 1999) (Esquiliano *et al.*, 2009)
Global INSR KO	INSR-	D15 ↔ D18/19 ↔	D15 ↔ Jz GlyT D18/19 ↔Jz GlyT		D15 ND D18/19 ↓10%	(Louvi *et al.*, 1997; Esquiliano *et al.*, 2009)
PI3K p110α (Pik3ca)	Kinase dead heterozygote Pik3ca-D933A	D16 ↓9%	D16 ↓Lz vol, FC vol, FC length, MBS vol, SA, diffusing capacity, ↑ BT, ↔ Jz	D16 ↑ glucose and System A transfer per unit SA, ↔ Glut1, Glut3, Snat1, Snat2, Snat4	D16 ↓19%	(Sferruzzi-Perri *et al.*, 2016)

		D19 ↓12%	D19 ↓ Lz vol, FC vol, FC length, Troph vol, SA, diffusing capacity, ↑ BT, ↔ Jz	D19 ↑ glucose and System A transfer per unit SA, ↑ Prl3b1, ↔ Glut1, Glut3, Snat1, Snat2, Snat4	D19 ↓11%	
Global decreased AKT signalling through increased PTEN	Prl2-/-	D17 ↓22%	D17 ↓Jz, GlyT and Lz	D17 ↓ passive transport	D17 ↓17%	(Dong *et al.*, 2012)
Global decreased AKT1 signalling	Pkba-/- (exons 4-8 deleted)	D17 ↓33% D19 ↓45%	D17 ↓ thickness, GlyT, Lz vessel density, length, area	D17 ↓ pAkt D19 ↓ total Akt, pAkt ↑ Akt2 and Akt3	D17 ↓17%	(Yang *et al.*, 2003; Yung *et al.*, 2008)
Global decreased AKT1 signalling	Pkba-/- (exon 1 deleted)	D18 ↓30%	D18 ↔ Lz and Jz Vd	D18 ↓ pAkt, ↔ p-Akt	D18 ↓22% weight and ↑ fetal loss	(Cho *et al.*, 2001; Kent *et al.*, 2012)
Global decreased MAPK signalling	Erk2-/-	D11 ↓	D11 ↓ Lz thickness, FC development	↓ MAPK signalling	D11 ↓ weight and ↑ fetal loss	(Hatano *et al.*, 2003)
Over-expression of IGF and downstream signalling						
Global IGF2 over-expression	Maternal Igf2r-	D16 ↑40% D18 ↑25%			D16 ↑40% D18 ↑40%	(Ludwig *et al.*, 1996; Louvi *et al.*, 1997)
Global IGF2 over-expression*	Maternal H19Δ13-	D15 ↑37% D16 ↑30%	D15 ↑ Jz GlyT D16 ↑ volume of all placental components, ↑ SA, diffusing capacity, ↔BT	D15 ↑ Akt1 ↔ pAkt, p-ERK1/2 D16 ↓ glucose transfer, passive permeability and Glut3, ↔Glut1, Snat1, Snat2, Snat4	D15 ↑30% D16 ↑12%	(Leighton *et al.*, 1995; Esquiliano *et al.*, 2009; Angiolini *et al.*, 2011; Church *et al.*, 2012)

		D18 ↑60% D19 ↑45%	D18 ↑Jz GlyT D19 ↑ volume of all placental components, SA, diffusing capacity	D19 ↓ glucose and System A transfer, passive permeability and Snat4, ↔ Glut1, Glut3, Snat1, Snat2	D18 ↑20% D19 ↑23%	
Global increased IGF2 and signalling via AKT	Double KO of maternal H19 and Pten +/-	D16 ↑65% D19 ↑80%	D16 ↑ Jz, GlyT D19 ↑ Jz, GlyT	↑ p-AKT and IGF2	D16 ↑31% D19 ↑31%	(Church *et al.*, 2012)
Global increased pAKT	Pten +/-	D16 ↑22% D19 ↑22%	D16 ↑ Jz, GlyT D19 ↑ Jz, GlyT	↑ p-AKT, ↔ IGF2	D16 ↑19% D19 ↑7%	(Church *et al.*, 2012)

*H19 null has biallelic expression of Igf2 combined with absence miR675 (encoded by H19)

Abbreviations: BT=barrier thickness, D=day, FC=fetal capillaries, GLUT/Slc2a=glucose transporter, GlyT=trophoblast glycogen cells, IGF1/Igf1 =insulin-like growth factor-1, IGF2/Igf2=insulin-like growth factor-2, Jz=junctional zone, LAT=L-type amino acid transporter, Lz=labyrinthine zone, MBS=maternal blood space, ND=not determined, PI3K=phosphoinositol 3-kinase, SA=surface area, SNAT/Slc38a= Sodium-coupled neutral amino acid transporter, Vd=volume density.

Search terms used: placenta, fetus, insulin-like growth factor, IGF, PI3K, ERK, MAPK, knock out, deficiency and/or transgenic

1547 **Table 3. The effect of maternal environmental challenge on fetal growth and placental structure, function and IGF signalling.**

Maternal manipulation	Species	Timing	Placental				Fetal weight	Reference
			IGF and signalling	size	morphology	function		
Nutrient restriction								
20% UN	Mouse	D3-D19	D16 ↑ IGF1R ↓ Igf2P0 and PI3K signalling D19 ↓ Igf2P0 and PI3K signalling	D16 ↓6% D19 ↓9%	D16 ↔Lz but ↓Jz and GlyT D19 ↓Lz (MBS and FC vols and SA), ↔BT	D16 ↓ Glut1 D19 ↑ System A amino acid transport, ↑ Glut1, Snat2, ↓Snat4	D16 ↔ D19 ↓13%	(Coan *et al.*, 2010; Sferruzzi-Perri *et al.*, 2011)
10-30% UN	Guinea pig	-D28	D35/40 ↓Igf2, ↔ Igf1	D35 ↓20% D60 ↓30%	D35 ↓ Jz volume ↔ Lz, but ↓ MBS, SA and ↑ BT D60: ↓ Lz volume, MBS, FC, SA, ↑ BT, ↔ Jz,		D35 ↓29% D60 ↓35%	(Roberts *et al.*, 2001; Olausson & Sohlstrom, 2003)
30% UN	Sheep	D22-D135	D135 ↔ Igf2	D135 ↓19% , altered placentome distribution			D135 ↓12%	(Osgerby *et al.*, 2002, 2004)
50% UN	Sheep	-D60-D30	D78 ↑ Insulin-IGF signalling (p-Akt and p-	D78 ↓29%	D78 ↑ vascularity		D78 ↔	(Zhu *et al.*, 2007b)

			ERK1/2)					
50% UN	Sheep	D28-D78	D78 ↑ Insulin-IGF signalling (p-ERK1/2, ↔pAkt) ↔ mTORC1 signalling D135 ↔	D78 ↓21% D135 ↔		D78 ↑Glut3, GLUT1, Fatp4 D135 ↑ Fatp4	D78 ↓26% D135 ↔	(Ma *et al.*, 2011)
UN gradual decrease to full food withdrawal	Sheep	D83-D90	D90 ↔ Igf2 D135 ↓Igf2	D90 ↓22% D135 ↔		D90 ↔ Glut1, Glut3 D135 ↔ Glut1, Glut3	D90 ↔ D135 ↔	(McMullen *et al.*, 2005)
UN 50%	Cow	D30-D125	D125 ↑ Insulin-IGF signalling (p-Akt and p-ERK1/2) D250 ↔	D125 ↓27% D250 ↓20%	D125 ↑ vascularity D250 ↔ vascularity		D125 ↔ D250 ↔	(Zhu *et al.*, 2007a)
70% UN	Baboons	D30-D165	D90 ↓ Igf2, IGF2R, ↑ IGF1R, ↔ Igf1 or IGF1 D120 ND	D90 ↔ D120 ↔		D90 ND D120 ↓ System A amino acid transport, ↔ system L amino acid transport, GLUT1 TAUT, SNAT1, SNAT2, SNAT4, LAT1,	D90 ↔ D120 ↔	(Li *et al.*, 2007; Pantham *et al.*, 2015) (Kavitha *et al.*, 2014)

			D165 ↓ insulin/IGF-I, MAPK (IRS-1, Akt S6K, ERK-1) and mTOR signalling	D165 ↓20%		LAT2 D165 ↓ System A and L amino acid transport, GLUT1, TAUT, SNAT2, LAT1, LAT 2	D165 ↓19%	
Low protein diets								
16% v 20% protein (0.80CT)	Mouse	D3-19	D16 ↔ Igf2, H19 D19 ↔ Igf2, H19	D16 ↑5% D19 ↑5%	D16 ↓ Lz/Jz ratio D19 ↓ Lz/Jz ratio	D16 ↑ glucose transport, Glut1, ↔ System A amino acid transport D19 ↓ System A amino acid transport, Snat4, ↔ glucose transport,	D16 ↔ D19 ↔	(Coan *et al.*, 2011)
8% vs 20% protein (0.40CT)	Mouse	D3-19	D16 ↑ total Igf2 ↔ Igf2P0, H19	D16 ↔	D16 ↔	D16 ↑glucose transport, Snat2, ↔ System A amino acid	D16 ↔	(Coan *et al.*, 2011)

			D19 ↔ Igf2, H19	D19 ↑4%	D19 ↔	transport D19 ↓ Snat1, Snat4, ↔ glucose and System A amino acid transport	D19 ↓9%	
9% vs 17% protein (0.53CT)	Rat	D1-22	D22 ↑Igf1 ↓Igf2 ↔Igf1r,Igf2r,Insr	ND			D22 ↓8%	(Nusken *et al.*, 2011)
6% vs 20% protein (0.30CT)	Rat	D1-21	D14 ↓ Lz Igf2, Insr in female and ↑ Lz IGF2, ↓ Igf1r in male D16 ↓ Lz Igf2 in female and male D21 ↓ Lz IGF2 in male and female	D14 ↓25% D18 ↓12% D21 ↔	D14 ↓Lz and Jz vol D18 ↓Lz vol, ↑ trophoblast stem cells and Lz sinuosoidal GiT, ↓ spongiotrophoblast and GiT cells, ↔Jz D21↔Lz ↓Jz		D14 ↓21.5 D18 ↓27 D21 ↓14%	(Gao *et al.*, 2012a; Gao *et al.*, 2012b, 2013)
4% vs 18% protein (0.22CT)	Rat	D2-21	D19 and D21 ↓ mTOR D21 ↓ PI3K signalling (p-Akt-T308)	D15-19 ↔ D21 ↓12.5%	ND	D19 and D21 ↓ Systems A and L amino acid transport, LAT1, LAT2, SNAT2, ↔ glucose transport,	D15-19 ↔ D21 ↓21%	(Jansson *et al.*, 2006{Rosario, 2011 #3227; Pantham *et al.*, 2016)

						SNAT4		
Obesogenic diets								
2.5-times fat	Mouse	-D28-D1	D13↓ Igf2, Mtor, ↔ Igf1 D18 ↓ Igf2, Igf2r, ↔ Igf1	D13 ↓20% D18 ↑15% in males, ↔ females	↔ Lz	D13 ↓ Snat1, Glut1, ↔ Cd36 D18 ↓ Cd36, ↔ Snat1, Glut1	D13 ↓28% D18 ↓15%	(Sasson *et al.*, 2015)
2.5-times fat	Mouse	-D28-D18	D1 3↑ Igf1r, ↓ Igf2, Igf2r, Mtor, ↔ Igf1 D18 ↓ Igf2, Igf2r, ↔ Igf1	D13 ↓20% in males D18 ↔ males or females	↔ Lz	D13 ↓ Snat1, Glut1, ↔ Cd36 D18 ↓ Cd36, ↔ Snat1, Glut1	D13 ↓25% D18 ↓25%	(Sasson *et al.*, 2015)
2.5-times fat	Mouse	D1-D18	D13 ↑ Igf1r, ↓ Igf2, Igf2r, Mtor, ↔ Igf1 D18 ↓ Igf2, Igf2r ↔Igf1	D13 ↓20% in males D18 ↑15% in males	↔ Lz	D13 ↓ Snat1, Glut1, Cd36 D18 ↓ Glut1, Cd36 ↔ Snat1	D13 ↓28% D18 ↓28%	(Sasson *et al.*, 2015)
5.3-times fat	Mouse	-D84-D19	D15 ↑ Igf2 and Igf2r male, ↔ female D19 ↔ Igf2 and Igf2r	D15 ↔ D19 ↔		D15 ↑ Lz Snat2 in male, ↑ Lz Snat4 in female D19 ↔	D15 ↔ D19 ↓8% in males	(King *et al.*, 2013)
6-times fat	Mouse	D1-15	D15 ↑ Igf1, ↓Irs1 in	D15 ↑7%	D15 ↔ Lz or	D15 ↓ Slc22a1,	D15 ↔	(Gallou-Kabani *et al.*,

			males, ↔ Igf2, Igf2P0, Igf2r, H19		vascularity	↑ Slc22a2 *sexually dimorphic response of placenta		2010; Gabory *et al.*, 2012)
2.5-times fat	Rat	D1-D21	D21 ↔ mTORC1 signalling	D21 ↔	D21 ↓ Jz		D21 ↓5%	(Mark *et al.*, 2011)
5-6-times fat	Rat	-D49-D21	D21 ↑ mTORC1 signalling, ↔ Insulin-IGF signalling (p-Akt or p-MAPK)	D21 ↔		D21 ↓ SNAT1, ↔ Systems A and L amino acid transport and LPL activity, SNAT2, SNAT4, GLUT1, GLUT3, GLUT9, FATP4, FATP6, LPL	D21 ↑7%	(Gaccioli *et al.*, 2013)
3-times fat and 5-times sugar diet	Mouse	-D42-D18	D18 ↓ mTORC1 signalling, ↔ Insulin-IGF PI3K (p-AKT, IRS1, PI3K-p85)	D18 ↔			D18 ↔	(Lager *et al.*, 2014)
4-times fat and 1.3-times sugar	Mouse	-D20-D19	D19 ↑ Insulin/IGF-PI3K (p-IRS1, p-Akt-T308) and mTORC1 signalling, ↔MAPK	D19 ↔		D19 ↑ Systems A and L amino acid transport, SNAT2, LAT1, GLUT1, GLUT3,	D19 ↑18%	(Diaz *et al.*, 2015; Rosario *et al.*, 2015; Rosario *et al.*, 2016)

						FATP6, ↔ SNAT4, LAT2, CD98, FAT/CD36, FATP2, FATP4		
3-times fat and 5-times sugar diet	Mouse	D1-D19	D16 ↑ Igf2, IgfP0, H19, Insulin/IGF-PI3K signalling (PI3K-p110α, p-Akt), ↓ INSR, ↔ mTORC1 or MAPK D19 ↑ Insulin/IGF-PI3K signalling (PI3K-p110α, p-Akt, p-MAPK), ↔ Igf2, Igf2P0, H19, INSR or mTORC1	D16 ↓11% D19 ↓8%	D16 ↓Lz FC ↑BT D19 ↓Lz, MBS, BT, SA and ↓GlyT	D16 ↑ glucose and System A amino acid transport, Glut3, Snat2 D19 ↑ FATP1, ↔ glucose and System A amino acid transport	D16 ↓9% D19 ↔	(Sferruzzi-Perri *et al.*, 2013)
50% greater food intake	Sheep	-D60-D135	D70-75 ↓ p-IRS1, p-mTORC1, p-MAPK in the arterial tissues, ↔ INSR, IGF1R D165 ND	D70-75 ↓22% D165 ↔	D70-75 ↑ arteriole diameters, ↓ vessel density D165 ↔	D70-75 ↑ Fatp1, Fatp4, Cd36, Lpl D165 ↑ GLUT3, FATP1, Fatp4, Cd36, ↔ Lpl	D70-75 ↑20-26% D165 ↔	(Zhu *et al.*, 2009; Ma *et al.*, 2010; Zhu *et al.*, 2010; Tuersunjiang *et al.*, 2013)
Hypoxia								
13%	Mouse	D1-D19	D19 ↑ Insulin-IGF	D19 ↑10%	D19 ↑ Maternal	ND	D19 ↓12% weight and	(Matheson *et al.*,

			(↑ p-Akt) and mTORC1 signalling		arterial and venous blood space		litter size	2015)
13% hypoxia	Mouse	D11-16	D16 ↓ Igf2, ↔ Igf2P0, altered p-Akt (depending on site phosphorylated)	D16 ↔	D16 ↑ Lz ↑ MBS, trophoblast vol, SA exchange	D16 ↔ System A amino acid amino acid or glucose transport, Gluts and Snats	D16 ↔	{Higgins, 2015 #5541
13% hypoxia	Mouse	D14-19	D19 ↑ Igf2, Igf2P0, altered insulin-IGF signalling (↓ INSR, IGF1R, PI3K-p85α, PI3K-p110α but ↑ p-Akt)	D19 ↔	D19 ↑ FC volume and density, ↓ BT	D19 ↑ glucose transport, Snat1, ↔ System A amino acid amino acid transport	D19 ↓5%	(Higgins *et al.*, 2015)
12% Hypoxia	Mouse	D14.5-18.5	D18.5 ↓ Igf2r and Igf2, Igf1r in females	D18.5 ↔	D18.5 ↓Lz blood space, ↑tissue in females	D18.5 ↓ Glut1, ↑ Snat1 in females, ↔ Glut3	D18.5 ↓6.5%	(Cuffe *et al.*, 2014)
10% hypoxia	Mouse	D14-19	D19 ↓ Insulin-IGF signalling (↓ INSR, IGF1R, PI3K-p85α, PI3K-p110α and p-Akt), ↔ Igf2, Igf2P0	D19 ↔	D19 ↓ Lz vd, MBS volume, SA exchange, ↑ Jz vd, trophoblast vol and BT	D19 ↓ System A amino acid transport, ↔ glucose transport but altered uterine artery vasoreactivity	D19 ↓21%	(Higgins *et al.*, 2015; Skeffington *et al.*, 2015)

Endocrine disruption								
Corticosterone 83μg/g/day	Mouse	D11-D16	D16 ↓ p-Akt, ↔ Igf2, Igf2P0, INSR, IGF1R, mTORC1 signalling	D16 ↓6%	D16 ↓ FC vol and Vd, ↑ MBS and Troph Vd, ↔ SA, BT	D16 ↓ Glut1, Glut3, Snat1, Snat2, ↔ glucose or System A amino acid transport and Snat4	D16 ↓7%	(Vaughan *et al.*, 2012; Vaughan *et al.*, 2015)
Corticosterone 81μg/g/day	Mouse	D11-D19	D19 ↓ mTORC1 signalling, ↔ Igf2, Igf2P0, INSR, IGF1R, p-Akt	D19 ↓12%	D19 ↔ FC, MBS, Troph, SA, BT	D19 ↓ glucose and System A amino acid transport, ↑ Snat1, ↔ Glut1, Glut3, Snat2, Snat4	D19 ↓19%	(Vaughan *et al.*, 2012; Vaughan *et al.*, 2015)
Dexamethasone 24μg/kg/day	Mouse	D13-D16	D16↓ MAPK1 D18 ↔ MAPK1 D16 and D18 ↔ Igf2	D16 ↓20% female only D18 ↔	D16↓Jz area female only D18 ND	D16 and D18 ↔ Glut1, Glut3, Snat1, Snat2, Snat4	D16 ↓20% D18 ↔	(Cuffe *et al.*, 2011)
Dexamethasone 24μg/kg/day	Rat	D13-D20	D20 ↓pAkt in Jz	D20 ↓50%		D20 ↓ Prls in Jz, ↑ Prls in Lz	D20 ↓22%	(Ain *et al.*, 2005)
Diabetes via streptozotocin administration neonatally	Rat		D20 ↑ Igf1, Igf2, Igf2r, IGF1R kinase and autophosphorylation activity, ↔ Igf1r, Insr	D21 ↑22%	D21	D21↑ glycerol and FFA release	D21 ↑13%	(Hauguel-de Mouzon *et al.*, 1992; Martinez *et al.*, 2008; White *et al.*, 2015)

Diabetes via streptozotocin administration 1 week before mating	Rat	-D7-D21	D21↓ Insr, Irs1, Igf2, Igf2r, ↔ Irs2, Igf1r	D21 ↑22%	D21↑ Lz, ↑ lacunae	D21↓ Glut1,↑ Lpl, ↔ Glut3, Snat2, Snat4, Lat1	D21 ↑5% or ↔	(Cisse *et al.*, 2013)
Insulin resistance via heterozygous p110α deficiency	Mouse		D16 ↓ PI3K signalling D19 ↓ PI3K signalling	D16 ↔ D19 ↑15%	D16 ↓ Lz Troph vol, ↓ BT D19 ↑ Jz vol ↑ SA diffusing capacity	D16 ↓ glucose transfer, Snat1, ↔ System A amino acid transfer, Glut1, Glut3, Snat2, Snat4 D19 ↓ glucose transfer, Glut1, Snat1, Snat2, Prls, ↔ System A amino acid transfer, Glut3, Snat4	D16 ↔ D19 ↔	(Sferruzzi-Perri *et al.*, 2016) *Depends on fetal genotype
Other manipulations affecting conceptus growth								
Restriction of utero-placental blood flow								
Uterine ligation	Mouse	D18	D20 ↓ Igf1, Igf2	D20 ↔	D20 ↓ Lz depth, vol,	D20 ↓ Slc5a9,	D20 ↓11%	(Habli *et al.*, 2013;

					vessel area	Slc7a10, 4F2hc, Lat1, Lat2, Snat2, GLUT1, GLUT8, ↑ Snat1, ↔ GLUT3, GLUT9		Jones *et al.*, 2013; Jones *et al.*, 2014)
Uterine ligation	Rat	D17	D20 ↓ Igf2	D20 ↓8%			D20 ↓20%	(Price *et al.*, 1992)
Uterine ligation	Rat	D18 or D19	D20 ↓ IGF1R, ↔ INSR	D20 ↔ or ↓25%	D20↑ diameter, ↔ Lz vd	D20 ↓ GLUT1, ↔ GLUT3	D20 ↓7% or 27% weight and ↓ litter size	(Das *et al.*, 1998; Reid *et al.*, 2002; Wlodek *et al.*, 2005)
Uterine ligation	Rat	D19	D22 ↓ Igf1, ↔ Igf2, Insr, Igf1r, Igf2r	ND			D22 ↔	(Nusken *et al.*, 2011)
Uterine ligation	Guinea pig	D30	D55-60 ↔ Igf1, Igf2	D55-60 ↔ or 37%		D55-60 ↓ System A amino acid transfer, ↔ glucose transfer	D55-60 ↓7% or 38%	(Jansson & Persson, 1990; Carter *et al.*, 2005)
Placental embolism	sheep	D113-120	D131 ↓ IGF1R, ↔ IGF-I	D131 ↓30%			D131 ↓21%	(Bloomfield *et al.*, 2002a; Shaikh *et al.*, 2005)
Placental embolism	sheep	D103-109	D131 ↔ Mtor	D131 ↓43%		D131 ↓ Glut1, Slc7a1, Slc7a8, ↔ Glut3, Glut4, Snat4, Slc7a5	D131 ↓20%	(Wali *et al.*, 2012)

Uterine carunclectomy	sheep	-D70	D130-134 ↑ Igf2, ↔ Igf1, Igf1r,Igf2r	D130-134 ↓ 30-40%	D130-134 altered distribution of placentome types and ↓ placentome number but ↑ individual weight of placentomes, trophoblast and maternal capillary volume and SA of placentomes	D130-134 ↓ Fatp4, ↔ Glut1, Glut3, Glut4, Slc7a1, Slc7a5, Snat1, Snat4, Fatp1, Cd36, Fabp5	D130-134 ↓26%	(Zhang *et al.*, 2016b)
Hyperthermia	Sheep	D39-D125	D55 ↑ IGF2 D90 ↑ IGF1 , p-mTORC1, ↓ p-Akt, ↔ MAPK D135 ↑ pAkt, p-MAPK dys-regulated mTORC1 signalling (↑ p-mTORC1 but ↓ p-p70)	D55 ↔ D90 ↓24% D135 ↓58%		D135 ↑ Slc7a5, Slc7a8, uterine blood flow, trans-placental oxygen diffusion, ↓ branched amino acid and glucose transport,	D55 ↔ D90 ↔ D135 ↓47%	(Thureen *et al.*, 1992; Ross *et al.*, 1996; Anderson *et al.*, 1997; Regnault *et al.*, 2003; de Vrijer *et al.*, 2004; Regnault *et al.*, 2005; de Vrijer *et al.*, 2006; Regnault *et al.*, 2007; Arroyo *et al.*, 2009; Arroyo *et al.*, 2010)

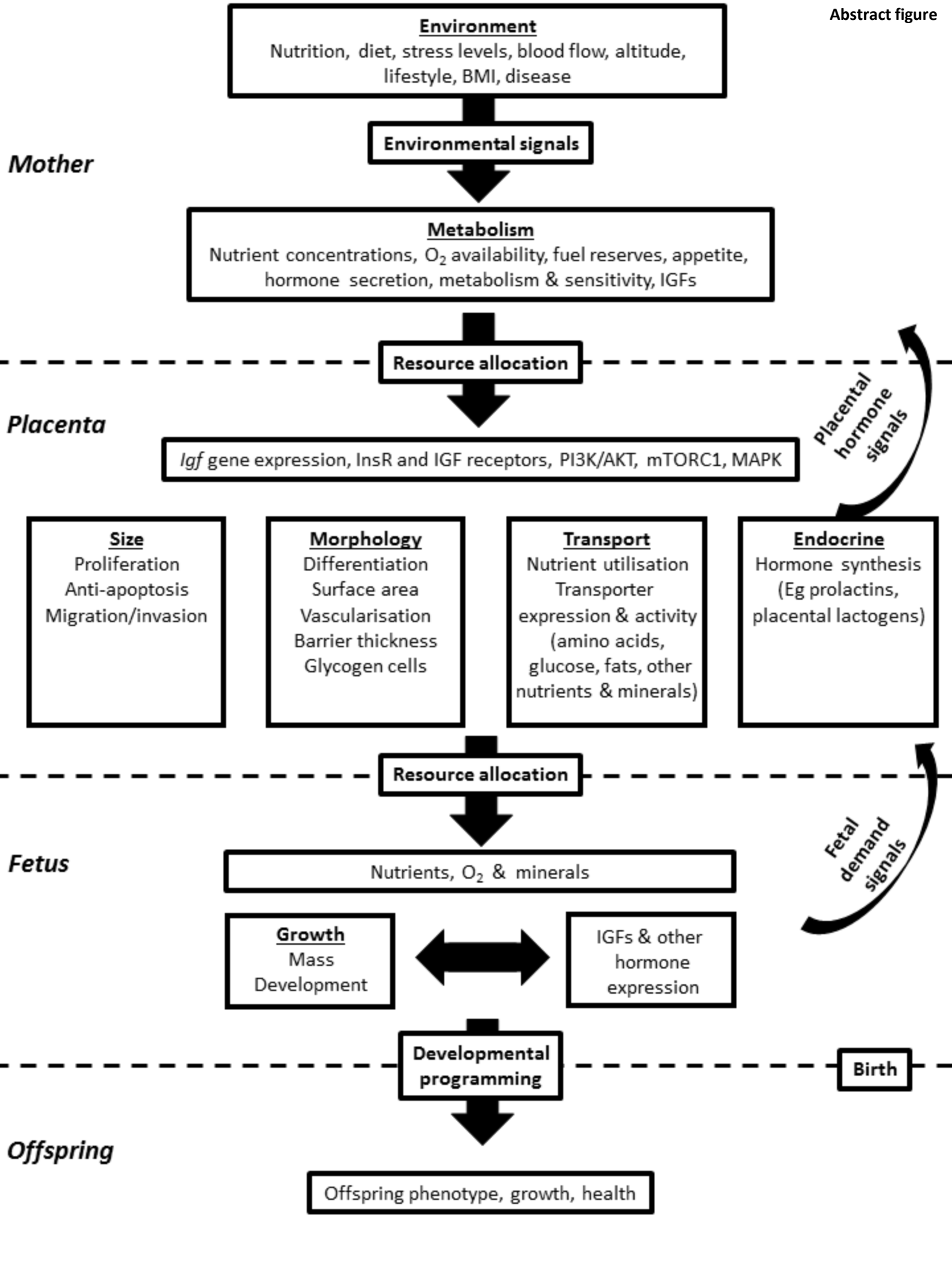
						↔ utero-placental oxygen uptake		
Alcohol consumption	Rat	-D4-D4	D20 ↓ Lz Igf1, and Lz Igf1r in males, ↔ Igf2 ↑ Jz Igf2, ↔ Jz Igf1, Lz or Jz Igf2r	D20 ↔	D20 ↑ length and width, ↓ Lz and ↑ Jz and ↑ GlyT in females	D20 ↓ Lz Snat2, ↔ Lz Snat1, Snat4, Glut1, Glut3 and ↓ Jz Glut1 in males, ↑Jz Glut1 in females	D20 ↓7%	(Gardebjer *et al.*, 2014)

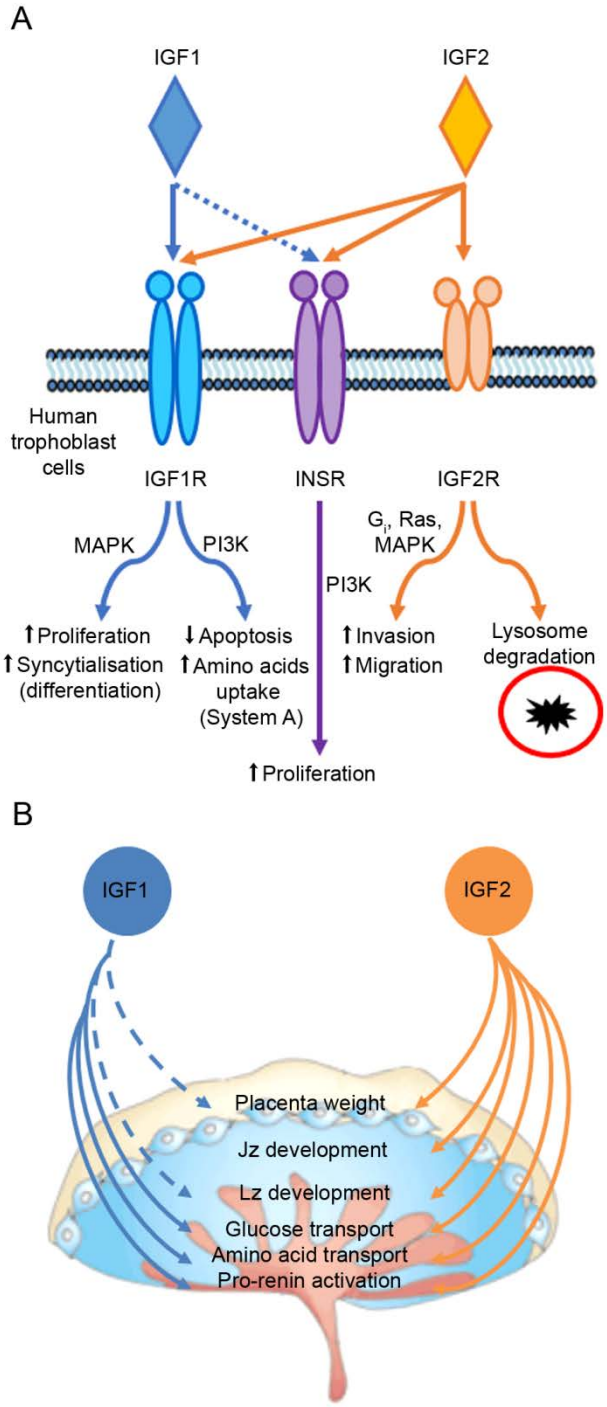
Gestational age: mouse ~20 days, rats ~23 days, guinea pigs ~70 days, sheep ~150 days, cows ~283 days, baboons ~183 days.

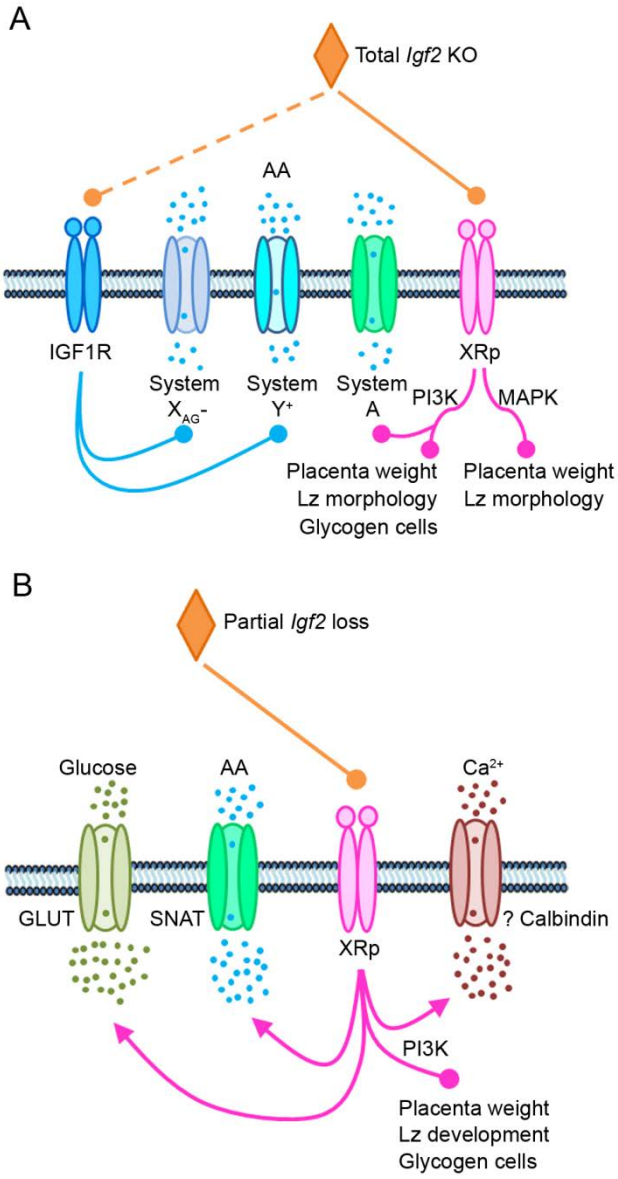
Abbreviations: BT=barrier thickness, D=day, FATP=fatty acid transport protein, FC=fetal capillaries, GLUT=glucose transporter, GiT- giant trophoblast cells, GlyT- trophoblast glycogen cells, IGF1/Igf1=insulin-like growth factor-1, IGF2/Igf2=insulin-like growth factor-2, Jz=junctional zone, LAT=cationic amino acid transporter, LPL=lipoprotein lipase, Lz=labyrinthine zone, MAPK/ERK=mitogen activated kinase, MBS=maternal blood space, mTOR=mechanistic target of rapamycin, p=phosphorylation, PI3K=phosphoinositol 3-kinase, Prl=prolactin-related hormone, SA=surface area, SNAT/Slc38a= Sodium-coupled neutral amino acid transporter, UN=undernutrition; vol=volume, vd=volume density.

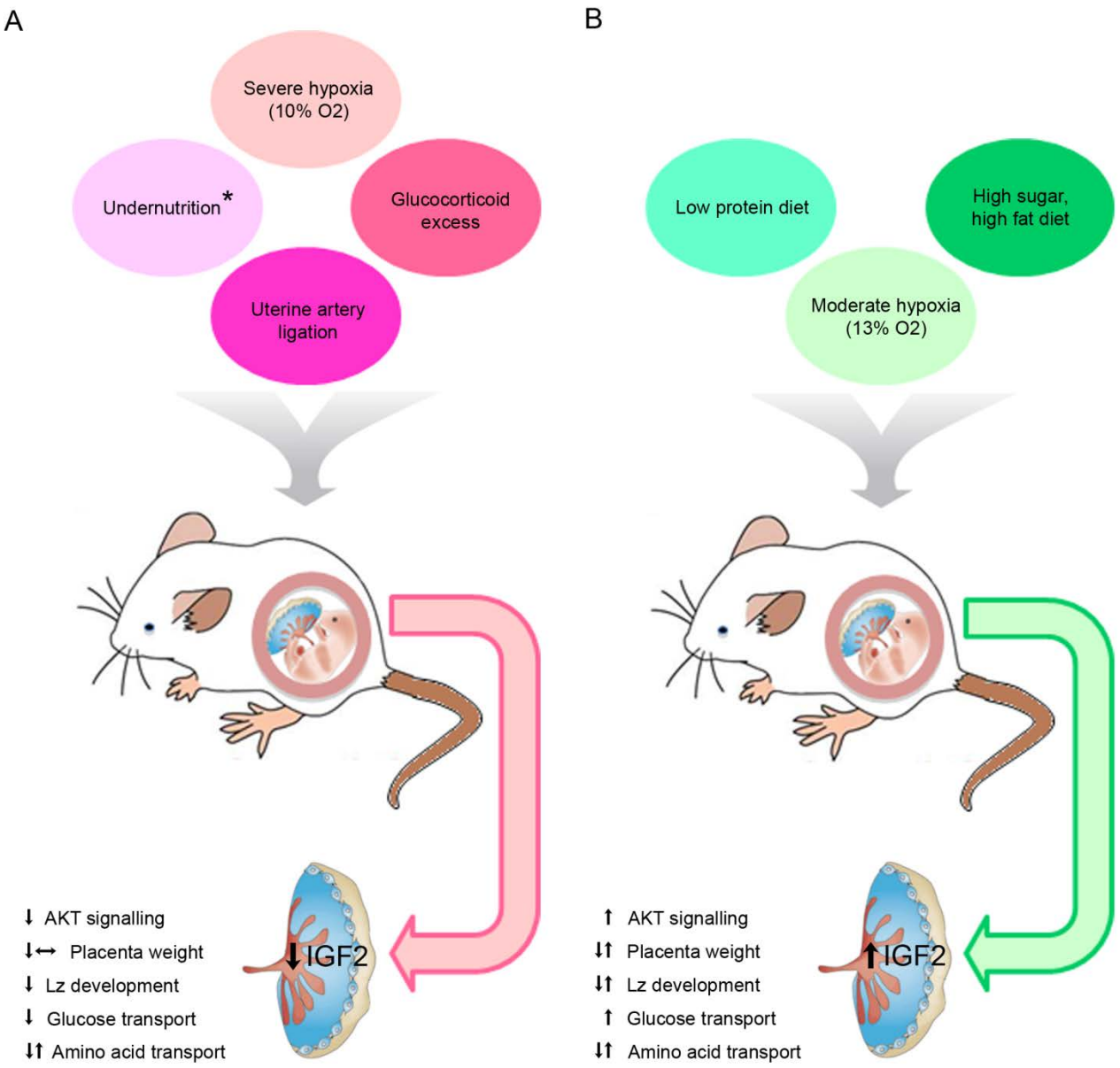
Search terms used: placenta, fetus, insulin-like growth factor, IGF, nutrient restriction, undernutrition, low protein diet, high sugar, high fat, obesogenic, IUGR, PI, hypoxia, uterine ligation, corticosterone, dexamethasone carunclectomy, heat stress and/or diabetes.

Abstract figure




A
IGF1
IGF2
Human trophoblast cells
IGF1R
INSR
IGF2R
MAPK
PI3K
PI3K
G_i, Ras, MAPK
↑Proliferation
↑Syncytialisation (differentiation)
↓Apoptosis
↑Amino acids uptake (System A)
↑Invasion
↑Migration
Lysosome degradation
↑Proliferation
B
IGF1
IGF2
Placenta weight
Jz development
Lz development
Glucose transport
Amino acid transport
Pro-renin activation


A
Total *Igf2* KO
AA
IGF1R
System X_{AG}^-
System Y^+
System A
XRp
PI3K
MAPK
Placenta weight
Lz morphology
Glycogen cells
Placenta weight
Lz morphology
B
Partial *Igf2* loss
Glucose
AA
Ca^{2+}
GLUT
SNAT
XRp
? Calbindin
PI3K
Placenta weight
Lz development
Glycogen cells


A
Severe hypoxia (10% O2)
Undernutrition*
Glucocorticoid excess
Uterine artery ligation
↓ AKT signalling
↓↔ Placenta weight
↓ Lz development
↓ Glucose transport
↓↑ Amino acid transport
↓IGF2
B
Low protein diet
High sugar, high fat diet
Moderate hypoxia (13% O2)
↑ AKT signalling
↓↑ Placenta weight
↓↑ Lz development
↑ Glucose transport
↓↑ Amino acid transport
↑IGF2